THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. 108—No. 3

Issued June 1, 1934

BALTIMORE, U. S. A.

1934

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

Made in United States of America

THE AMERICAN JOURNAL OF PHYSIOLOGY

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 108 JUNE 1, 1934 No. 3

THE EXPERIMENTAL PRODUCTION OF EDEMA AND ELEPHANTIASIS AS A RESULT OF LYMPHATIC OBSTRUCTION

CECIL K. DRINKER, MADELEINE E. FIELD, AND JOHN HOMANS

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication February 26, 1934

One of the oldest methods for the study of function consists in ablation of an organ, system, or part in a normal animal, with subsequent observation of the changes so induced. Efforts to study the lymphatic system of mammals by such methods have been fairly numerous and uniformly unsuccessful. As an example of the difficulties encountered, one may cite the work of Reichert (1926). He divided all the tissues in the middle of the thigh in a series of dogs, with the exception of the femur, femoral artery and vein, and sciatic nerve. The various structures were at once reunited, and normal healing followed. Such an operation divided the lymphatics of the thigh practically completely. On the second day after operation the leg was swollen below the incision, and swelling continued to increase until the fourth or fifth day, when it began to subside and was entirely gone by the seventh or eighth day. Injections showed that lymphatics began to cross the healing incision on the fourth day, and by the eighth day a wholly adequate lymph circulation was re-established. Our experience with surgical attempts to interrupt or destroy lymphatics in dogs has given equally transitory effects. One may remove the iliac and popliteal lymph nodes, ligating the lymphatics which enter and leave them, and to these interruptions of lymphatic continuity a section of Reichert's type may be added, but so great is the impetus for restoration of lymph drainage that permanent lymphedema cannot be maintained.

Against such experimental failures stand the numerous cases of lymphatic obstruction with edema and elephantiasis which occur in the tropics following infection by Wuchereria Bancrofti and, more rarely, in temperate climates following a variety of experiences.

Drinker and Field (1933), in a preliminary account of the experiments which follow, pointed out that the surgical attempts to produce permanent

lymphedema were single attacks upon the lymphatic system, whereas lymphedema in man was apparently the result of a continuous process or of a repeated series of attacks. The experiments which follow show that the lymphatics in the leg of the dog can be blocked, provided one makes use of methods simulating the continued action of chronic disease. This blocking results in lymphedema and elephantiasis. When it is well established, the animals are prone to have spontaneous attacks of chills and fever, with redness, heat, and swelling in the affected part, just as is so often the case in human lymphedema and elephantiasis. During these attacks a hemolytic streptococcus has been grown from the edema fluid with unvarying regularity. The organism cannot be obtained between attacks.

The experiments as a group have made it possible to study the composition of the edema fluid, the development of elephantiasis, and the bacteriological and immunological features of the attacks of chills and fever with acute disturbance in the affected leg.

Experiments. Large lymphatics—draining trunks—are less numerous in the dog than in man, and this is a fortunate circumstance for the establishment of blockage. In the hind leg large lymphatics can be found with the great saphenous vein on the dorsum of the foot. These vessels, in the main, follow the external saphenous vein and run into the popliteal lymph node. Efferent vessels leave this node and follow the femoral vessels to Poupart's ligament and the iliac vessels to the inferior vena cava. The internal saphenous vein is accompanied by a similar group of large lymphatics which do not enter the popliteal node. They join the external group where the internal saphenous vein enters the femoral vein, and follow the great vessels into the pelvis to enter the iliac lymph nodes. There is a free communication between the large lymphatics of both legs at the level of the iliac nodes. The main trunks are joined by collecting vessels from the abundant plexus of lymphatics in the skin, and by a smaller number of vessels from the deeper structures in the leg. Figure 1, A, B, and C, is a diagrammatic representation of the main trunks, the structures with which we are concerned in blocking the lymph drainage from the leg.

The technique of accomplishing blockage is quite simple but requires persistence. Under a general anesthetic, and in our experience nembutal (sodium-ethyl (1 methyl-butyl) barbiturate) is ideal, a small incision is made over a large lymphatic. In figure 1-A, dog 1, the first incision was upon December 12, 1932, at the level of the ankle. Through this incision lymphatics of both the internal and external group were exposed and cannulated with small quartz cannulae. Through these cannulae two injections were given centrally—in both cases slowly and with as little pressure as possible, so as to secure a maximum degree of deposition in lymph nodes and lymphatics. The first injection consisted of a water suspension of crystalline silica, with an average particle size of 1 micron. This material,

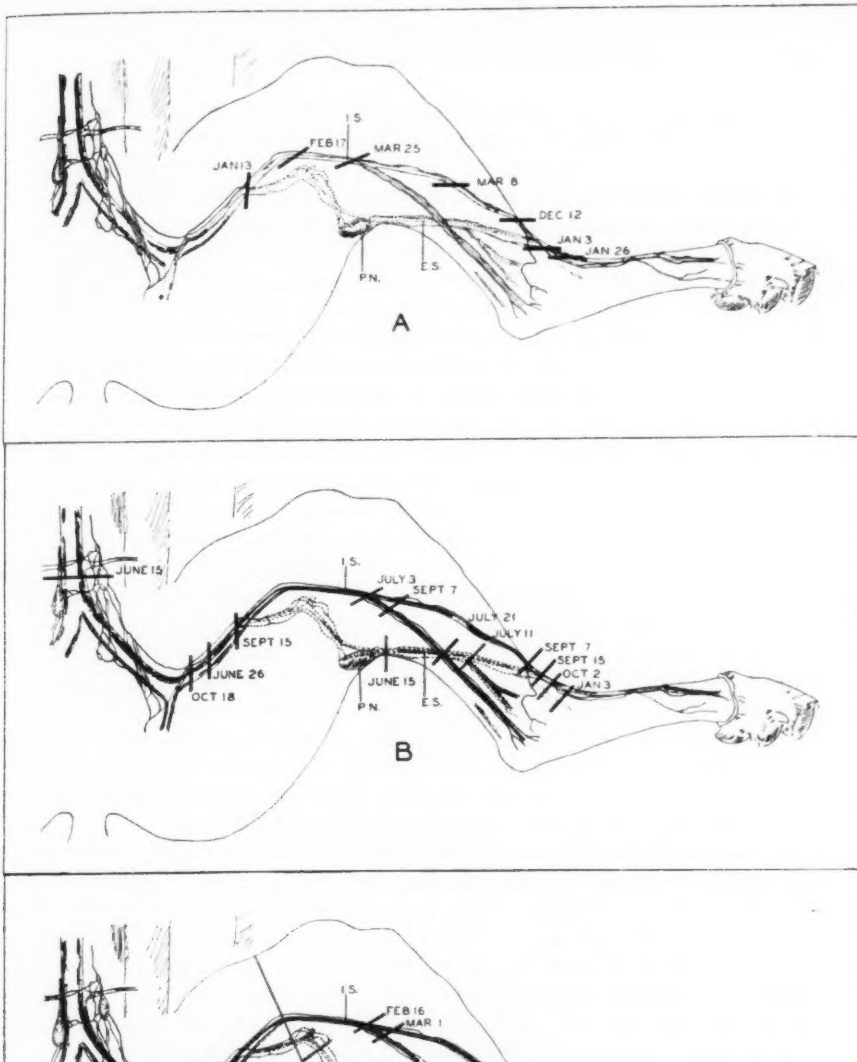

Fig. 1. Lymphatics in the hind leg of the dog, with sites of successive injections of quinine hydrochloride and silicon dioxide. *A*, dog 1; *B*, dog 2; *C*, dog 3. *P.N.*, popliteal lymph node; *E.S.*, external saphenous vein; *I.S.*, internal saphenous vein. Leg viewed from the inner side.

when deposited in lymph nodes, causes the appearance of very characteristic large, pale cells undoubtedly arising from the endothelial reticulum and the lining of the sinuses of the node. These cells accumulate in the sinuses in large enough numbers to obstruct lymph flow, and eventually become foci of fibrous tissue formation. The process is identical with that seen in the lungs following inhalation of silicious dusts and, indeed, the silica employed in our experiments had a noteworthy reputation as the cause of lung fibrosis in workmen. In the experiment of December 12, 1932, 2 to 4 cc. of the silica suspension were injected, and this was followed by a similar volume of 2 to 2.5 per cent quinine hydrochloride in water. This substance has been used to sclerose varicose veins. It is destructive to the lymphatics, is entirely painless, and causes no systemic effects if given slowly. At the present time it is our practice to inject 3 cc. of the quinine solution during 10 minutes' time. This is repeated after 30 minutes, and again if possible after another 30 minutes. It is the aim to accomplish as intense a contact between the quinine solution and the structures entered by it as is possible, and this will not be attained if the solution is pushed through rapidly.

In dog 1, whose operative course is covered diagrammatically in figure 1-A, a second similar injection was made on January 3, 1933, a third on January 13, 1933, and so on until March 25, 1933. The sites of the injections are indicated by the heavy line accompanying each date. Two things are apparently accomplished by the successive injections. First of all, the lymphatic apparatus is loaded with particles of crystalline silica which continue to produce a reaction peculiarly potent for causing lymphatic obstruction; and, second, each new injection wipes out collateral paths and new paths which might result in satisfactory lymph drainage.

It is possible to produce a fair degree of lymphatic obstruction by operating upon one leg only, but in our experience better results are obtained if both legs receive injections. Thus, in dog 1, figure 1-A, four injections were made in the right leg prior to the first in the left, and five further injections were made in this leg before March 25, 1933 when no more large lymphatics could be found in the left leg. Upon this date, several small vessels were tied and cut. In dog 2, figure 1-B, a slightly different course was followed. On June 15, 1933 a low left paramedian abdominal incision was made. The iliac lymphatics and nodes having previously been filled with 2 per cent trypan blue (White, Field and Drinker, 1933), it was easy to ligate lymphatics and remove the nodes on both sides. Before closure of the abdomen an incision just below the popliteal space permitted cannulation of an afferent lymphatic to the popliteal node. Through this cannula, 2 cc. of silica solution and 4.5 cc. of 2.5 per cent quinine hydrochloride solution were injected. There was no leakage of either fluid in the abdomen. The points of subsequent injection are given in the diagram, figure 1-B, the last being low down in the ankle on January 3, 1934. On

October 18, 1933, no lymphatics were found and no injection made. Between June 26 and October 2, 1933, five injections were made in the opposite leg.

Figure 1-C, dog 3, is self-explanatory. The silica and quinine injections began on January 19, 1933 and ended on May 24, 1933. On June 21, 1933, a circular incision through the skin and to the muscle was made in the middle of the thigh. One large lymphatic was found on the inner side of the leg. This was tied and cut. A circular incision of this type is not effective in blocking lymphatics. It does, however, enable one to find large lymphatics which have developed in anomalous regions as a result of blockage.

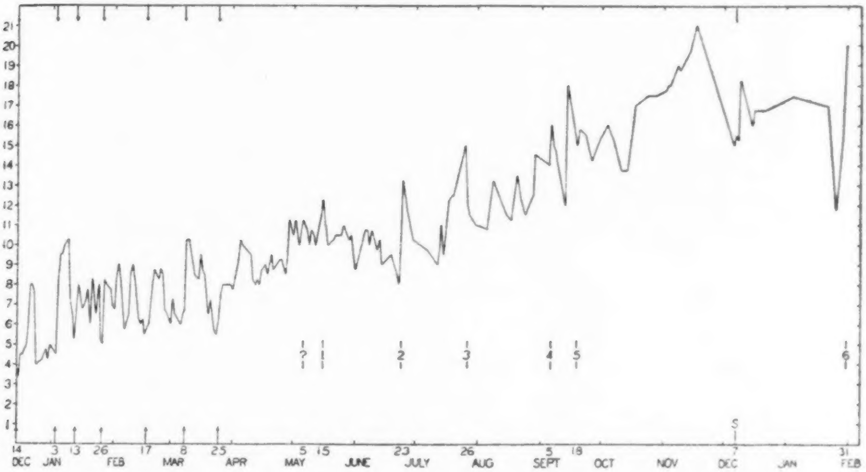

Fig. 2. The degree of swelling in the left hind leg of dog 1. *Abscissae*, days; *ordinates*, degree of swelling over normal. Arrows on the line of the abscissa indicate the days of operation and injection. The question mark (?) and figures *1*, *2*, *3*, *4*, *5*, and *6* indicate attacks of chills and fever with acute generalized inflammation of the leg. *S* on the abscissa indicates a subcutaneous injection of streptococci isolated from this animal during the second febrile attack on June 23. Weight of dog, 41 kgm.

At the present time it is our practice to make the first injection high in the leg and to work toward the foot. On each occasion it is most important to inject slowly and in divided amounts, so as to deposit quinine solution and silica as widely as possible.

The rate and manner in which the legs shown in figure 1 swelled is brought out in figures 2, 3 and 4. These charts have been constructed as follows: Daily, or at frequent intervals, four measurements of the circumference of the leg have been made—the first at the base of the toes, the second half way to the ankle joint, the third at the ankle joint, and the

fourth 1 inch below the knee. Prior to the first operation the measurements on the left hind leg of dog 1 were $6\frac{1}{4}$ inches, $5\frac{1}{2}$ inches, $6\frac{1}{2}$ inches, and $6\frac{1}{4}$ inches, giving a total of $24\frac{1}{2}$ inches. On November 16, 1933, when the swelling of the leg was extreme, the measurements were 9 inches, 12 inches, 13 inches, and $11\frac{1}{2}$ inches, totalling $45\frac{1}{2}$ inches. If from this figure the normal total of $24\frac{1}{2}$ inches is subtracted, there remain 21 inches of excess circumference. The ordinates in figures 2, 3 and 4 represent repeated esti-

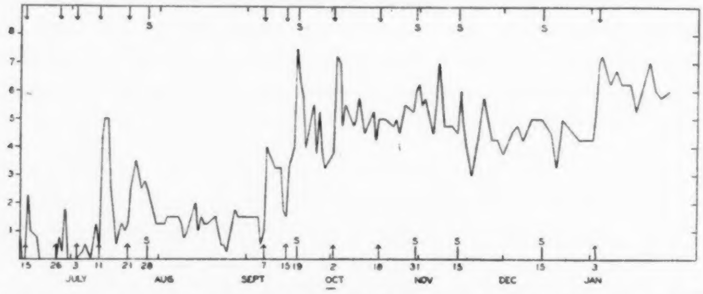

Fig. 3. The degree of swelling in the left hind leg of dog 2. Method of charting identical with figure 2. Weight of dog, 30 kgm.

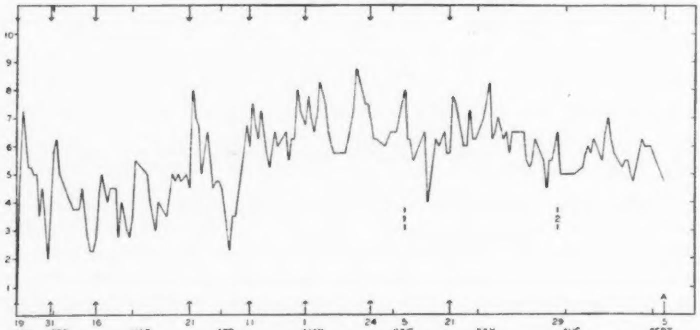

Fig. 4. The degree of swelling in the left hind leg of dog 3. Method of charting identical with figures 2 and 3. Weight of dog, 26.5 kgm.

mates of excess circumference, and the charts thus record the increase in the size of the leg over a normal standard obtained before beginning lymphatic obstruction.

In the case of figure 2, which deals with dog 1, the first operation and injection occurred on December 12, 1932. Owing to an error no measurements were made on that day or on the 13th. As a result of the injection the leg swelled markedly and continued to do so until December 22, when

it subsided abruptly and remained at a fairly uniform level until January 3, 1933, the date of the second injection. The successive injections permitted only partial subsidence of swelling, and though fluctuations are evident the general slope of the curve is upward. Figure 5 consists of four photographs of the hind legs of this animal. Owing to the fact that the right leg is also swollen, the contrast is not so great as the actual situation merits. The first photograph was taken on January 9, 1933, after two injections in the left leg; the second on March 17, 1933; the third on August 17, 1933; and the fourth on October 9, 1933. From December 12 until

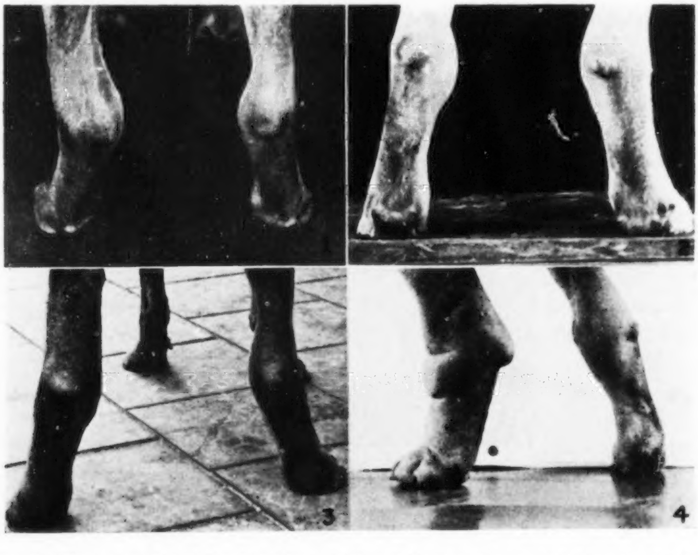

Fig. 5. Photographs of the hind legs of dog 1 during the period January 9, 1933 to October 9, 1933. *Number 1*, January 9, 1933; *number 2*, March 17, 1933; *number 3*, August 17, 1933; *number 4*, October 9, 1933.

May 5, the swelling recorded in figure 2 and in the first two photographs in figure 5 was the result of the injections alone.

On January 25, 1934, the dog had a day of intense muscular activity and excitement. During this period he scratched the outer side of his foot superficially, but sufficiently to permit free leakage of edema fluid. When he returned home about 9:30 in the evening, it was noticed that the left leg had lost greatly in size. This loss is shown by the drop in the curve (fig. 2) on January 26. At this time the leak had closed and edema fluid was easily obtained by puncture, but the total amount in the leg was much less and the swelling recorded on the 26th is due practically entirely to fibrous change.

On the day following injection of the sclerosing material, the leg may be somewhat warmer than normal and the edema fluid may show a slight increase in cell count. The animals have never shown persistent lameness, pain, nor evidence of infection. There is a transient period of direct chemical irritation which, as time passes, is coupled with more and more lymphatic block. After the first injection the edema is doughy and it is hard to obtain edema fluid by puncture. As the process is forced by subsequent injections, edema fluid accumulates plentifully and the subcutaneous tissue becomes thick and fibrous. The details of the pathological changes will be dealt with in another paper, since it has been possible to take biopsy material at frequent intervals.

It may be stated, however, that following lymphatic block, and what at their worst are very transient periods of sterile irritation, the part becomes characteristically elephantiatic. Infection is not necessary in order to

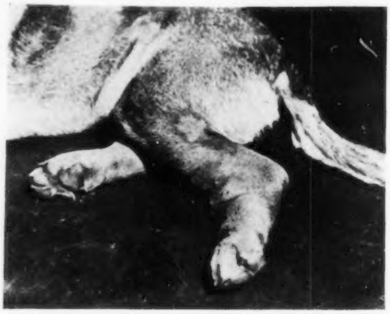

Fig. 6. Side view of the left hind leg of dog 1, January 19, 1934

bring this about, but if infection occurs the elephantiasis develops much more rapidly. An advanced condition is shown in figure 6. In the case of dog 1, whose swelling is charted in figure 2, there were six, and possibly seven, spontaneous attacks of acute generalized inflammation in the left leg. These took place on May 5, May 15, June 23, July 26, September 5 and September 19, 1933, and January 31, 1934. The attacks were never associated with any local lesion. They lasted about 48 hours and were similar clinically to those seen so frequently in human cases of lymphedema and elephantiasis. The leg became hot, red and tender, with no signs of localization. It swelled greatly and the edema fluid became heavily loaded with leucocytes. A hemolytic streptococcus was isolated from the edema fluid early in the attacks, but was never found between attacks. Since it has been possible to learn many details about these attacks they will be discussed in a later paper. It is necessary to mention them here since they are one of the consequences of lymphatic obstruction.

Just as it has been possible to follow the pathogenesis of elephantiasis, so too has it been easy to make repeated examinations of the edema fluid during the entire period of observation of each animal. Drinker and Field (1931) suggested that if the plasma proteins leak from the capillaries constantly, and this material can leave the tissues through the lymphatics alone, then, in the presence of lymphatic obstruction, the tissue fluid must develop permanent high concentrations of protein. This expectation has been realized. In the case of dog 1, the protein in the edema fluid reached 4 per cent in August, and has varied between 3.7 and 5.7 per cent ever since. The fluid clots. The albumin-globulin ratio is that of blood plasma, and on microscopic examination one invariably finds white cells which stain diffusely and have lost their nuclei. The tissue fluid has thus been converted into a solution containing something over half the concentration of the proteins in the blood, together with disintegrated and disintegrating cells Such a medium provides excellent growth of connective tissue cells *in vitro* and is, perhaps, the cause of the elephantiatic changes observed in our animals. A complete account of the chemical and cellular composition of edema fluid in such animals as dog 1 would require space beyond the possible limits of this paper, and will be found in a later communication.

Figure 3, constructed exactly as figure 2, gives the course of swelling in the left hind leg of dog 2. Arrows on the abscissa represent the injections which appear in figure 1-B. The letter *S* on the abscissa indicates subcutaneous injections of streptococci isolated from the edema fluid of dog 1 during attacks. These injections invariably caused a diffuse reaction in the leg, with a chill and high fever—a transient illness exactly similar to the spontaneous attacks encountered in dog 1 and in two other animals with lymphedema. It is of interest that permanent swelling of the leg was not established until September 15, and required seven injections. During the same period, five injections were given in the lymphatics of the right leg. This animal has had no spontaneous attacks such as have occurred in dog 1 and in dog 3, but he has had exactly comparable seizures due to the streptococcal injections in the left leg.

Figure 4, constructed as were figures 2 and 3, indicates the swelling of the left hind leg of dog 3, whose operative course is shown in figure 1-C. This animal leaked edema fluid during much of his course. These leaks are not caused by large fistulae, but come from minute points in the skin, often between the toes. They are quite painless and grossly very hard to see. In some animals they are so persistent and extensive as to keep the protein concentration of the edema fluid very low, and in such cases the permanent fibrotic enlargement of the part is not impressive. In the case of dog 3, the leakage was intermittent, sometimes stopping for a number of days. Between March 29, 1933 and July 12, the protein in the edema fluid varied between 2.17 and 2.95 per cent, and was usually over 2.5 per

cent. Since the swelling was not progressive the dog was killed on September 5, 1933 and autopsied.

DISCUSSION. Subcutaneous tissue fluid may move from the region in which it is formed in three ways. First of all, it may remain extravascular and diffuse through the tissues as a result of gravity. This sort of movement becomes very noticeable when gross edema is present, but any normal individual standing at attention for long periods loses fluid from the vessels in his legs, and after a time it is noticed that the feet and legs are swollen. The second possible pathway is offered by the lymphatic system. Proteinized tissue fluid enters lymph capillaries very easily and, on motion of the part or change of position, some of it is forced into larger vessels containing valves. It is then moved along through the ordinary lymphatic pathways and eventually reënters the blood stream. Added to these two methods of fluid movement there is a third, namely, reabsorption into the blood capillaries. This is unquestionably the main pathway by which extravascular water and salts are removed, but extravascular blood proteins apparently fail to return directly to the capillaries and somewhere or other must enter the lymph stream in order to reach the blood.

When the lymphatics are blocked, as in the experiments we have described, water and salts continue to pass in and out of blood capillaries, but extravascular protein cannot move from the part except by diffusion of the tissue fluid through the tissue spaces. On first establishment of lymph block, these spaces are extremely small and diffusion through them is slow, but as the part enlarges the tissue spaces become very noticeable even though there has been large new formation of fibrous tissue. Under these circumstances the tissue fluid follows gravity quite rapidly.

One of the first effects of lymphatic block is the appearance of varicose lymphatic trunks. These are seen as hugely dilated elements. When cannulated centrally lymph flows out of the cannula plentifully, indicating the incompetence of the valves. As blockage persists lymph capillaries in the skin and subcutaneous tissue dilate widely and their walls become somewhat thickened.

In spite of all these changes edema fluid would not accumulate in great quantity were it not for the fact that the leg is dependent so much of the time. With the dog upon his back, and the leg upright, the edema fluid drifts slowly up through the subcutaneous tissue, and through dilated lymphatics in the skin, until presumably it reaches the abdomen and back and finds itself in regions where there are unblocked lymphatics with competent valves. Through these it returns slowly to the blood stream. Elephantiatic change can occur as a result of repeated infections in regions that are not dependent (Stevens, 1933), but as a consequence of lymphedema, with at most a very slight element of inflammation, such change is confined to dependent parts where tissue fluid can accumulate and where

the part experiences a sustained change in the protein environment of the tissue cells. Tissue growth occasionally follows long periods of edema due to venous stasis or to nephrosis, but in such cases is usually localized to regions where there have been attacks of erysipelatous infection. Where edema fluids appear which are low in protein, tissue growth does not occur. An addition of protein, and possibly of disintegrating cells, is necessary and these changes are an essential accompaniment of inflammation. If attacks of inflammation are frequent the tissues involved experience equally frequent changes in tissue fluid, together with some degree of obliteration of lymphatic capillaries, making removal of the highly proteinized tissue fluid proportionally slow. The situation is very like that which is inevitable in simple lymphatic obstruction, where failure of removal of blood proteins and disintegrating cells from the tissue fluid results in a new fluid environment for the part affected.

Conclusions. When lymphatic drainage of the dog's hind leg becomes permanently impossible, three things happen which illustrate the normal constant function of the lymphatics:

1. There is progressive accumulation of edema fluid which eventually contains as high as 4 to 5 per cent of protein. This clots and has an albumin-globulin ratio similar to blood. In addition, the fluid contains cells of various sizes and in varying numbers. These, in the main, stain very poorly and seem to be undergoing a slow degeneration.

2. Overgrowth of connective tissue, and dilatation and thickening of lymphatic capillaries begin to be noticeable when lymphedema has been present for two months. These changes are those underlying elephantiasis.

3. Whatever may be the effect of acute lymphatic block in restraining acute infection (Menkin, 1931), there can be no doubt that when a part loses lymphatic drainage permanently, so that with each period of activity there is no movement of fluid from the tissues to the lymphatics out of the region, then there develops a surprising susceptibility to streptococcic infection. This susceptibility is all the more striking when one considers the difficulty of infecting dogs with streptococci.

The attacks of spontaneous infection which occur in the lymphedematous and elephantiatic legs of these dogs are entirely similar to those seen in human cases, both in the tropics and in temperate climates. Such periods of repeated infection intensify the changes in the tissue fluid which result from lymphatic obstruction alone, and it is not surprising that they have been considered the underlying cause of elephantiasis. They are not the essential cause, but they do accelerate the new growth of connective tissue.

SUMMARY

1. Three typical instances of lymphatic obstruction in the hind leg of the dog have been described. Similar changes have been produced in four other animals.

2. Obstruction has been brought about by repeated central cannulation of lymphatic trunks with injection of a suspension of crystalline silica and a 2.5 per cent solution of quinine hydrochloride.

3. Lymphedema developed after such injections and eventually became pronounced. The protein content of the edema fluid rose slowly to above 4 per cent.

4. With the establishment of lymphedema the subcutaneous connective tissue increased and the leg gradually became elephantiatic.

5. In the presence of chronic lymphatic obstruction the part became susceptible to attacks of acute infection. A hemolytic streptococcus has been isolated from the edema fluid early in these attacks. The seizures were exactly like the attacks which occur in human cases of lymphedema and elephantiasis.

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THE RÔLE OF PHOSPHOCREATINE IN THE FUNDAMENTAL CHEMICAL CHANGES IN CONTRACTING MAMMALIAN MUSCLE

JACOB SACKS AND WILMA C. SACKS

From the Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan

Received for publication February 24, 1934

The developments which have taken place in the chemistry of muscular contraction since the discovery of phosphocreatine by Fiske and Subbarow (3) in 1927 have led to a decided shift in the views concerning the rôle of this substance in the contraction process. Finding that phosphocreatine undergoes hydrolysis during contraction, when lactic acid is being produced, and that this hydrolysis liberates large quantities of base, these investigators postulated (4) that the function of this hydrolytic reaction was to buffer the muscle against the lactic acid. The work of Lundsgaard (7) in 1930 on muscles poisoned with iodoacetic acid, in which lactic acid formation is inhibited, led him to the view which is now current, that the hydrolysis of phosphocreatine is the immediate source of the energy for contraction. When such muscles contract in the absence of oxygen, the phosphate portion of the phosphocreatine molecule does not appear as inorganic phosphate, but instead becomes converted to a mixture of hexosephosphates. Lundsgaard postulated that the formation of hexosephosphate from glycogen and some of the phosphoric acid resulting from the hydrolysis of phosphocreatine was the means of furnishing energy for the resynthesis of the remaining phosphoric acid back to phosphocreatine, in which form it would again be available for the energy for contraction.

In the previous papers of this series (15), evidence was presented that phosphocreatine has a dual function in muscle. So far as the hydrolytic reaction is concerned, the data are in full agreement with the hypothesis of Fiske and Subbarow. The second reaction of phosphocreatine, conversion to hexosephosphate, was shown to serve the double purpose of furnishing energy for anaerobic work and of buffering against lactic acid. The formation of this latter substance from glycogen is the other important source of energy in the absence of oxygen.

In the muscles of the rabbit, upon which species the first work was done, the amount of phosphocreatine hydrolysis is inadequate, were this the sole buffering mechanism present, to neutralize even half of the lactic acid

formed. In the rat the hydrolytic reaction is quantitatively more important, amounting to two-thirds of that necessary to neutralize all the lactic acid formed. It was pointed out that the additional buffer liberated by the formation of hexosephosphate was adequate, in this species, to make up the deficiency. Hence there is no need to assume that the muscle fiber undergoes any change in pH as a result of contraction. However, the total amount of base liberated by the two reactions of phosphocreatine in the rabbit is significantly less than the quantity calculated to be necessary to prevent a change in pH.

A possible explanation of this difference presents itself if the diets of the two species are considered. The rabbit normally has a diet in which base is in excess and the rat one in which acid-forming substances predominate. Hence the rabbit should be able to store base in the tissues, presumably in the form of the alkali-protein complex postulated by Meyerhof and Lohmann (12). If some base is liberated from this material, then the hydrolysis of only a small quantity of phosphocreatine would be necessary to maintain the pH of the muscle fiber constant during contraction.

If this explanation of the difference between the two species is valid, and if it should be possible to deplete the tissues of the rabbit of their supposed excess of alkali, the muscles would then be in the same state as those of the rat are normally. Under these conditions, the amount of phosphocreatine hydrolyzed should be appreciably greater in proportion to the amount of lactic acid formed.

When the experiment was tried, it was found to be quite difficult to remove the excess base from the tissues of adult rabbits by dietary means. However, if young, growing, animals were placed on a diet deficient in base, they could apparently be kept from building up such a tissue reserve. The stock diet for rabbits in this laboratory consists of oats and alfalfa hay, with some lettuce at intervals. The hay contains an excess of potassium and the rabbits generally eat enough of it so that the urine is alkaline to litmus. Oats, on the other hand, contain relatively large amounts of sulfur and phosphorus which appear in the urine as sulfates and phosphates. Since there is relatively little base in the diet, the urine of animals fed only oats is strongly acid to litmus. Albino rabbits about three or four months old are apparently unable to store alkali on such a diet.

The experimental procedure was to place rabbits of about the same age and weight on the two diets, and after a suitable interval—ten days is generally sufficient—perform stimulation experiments, as was done previously. The animals were anesthetized with sodium pentobarbital and ether. It was found that younger animals require relatively less pentobarbital than adult ones. Hence the dose used was 80 or 90 per cent of that used previously, depending on the size of the animal. Both gastrocnemius muscles were dissected free, leaving the blood supply and innerva-

tion intact. One muscle was then frozen *in situ* as a resting control, using a mixture of powdered solid carbon dioxide and ether. The other was then tetanized through the nerve for five or fifteen seconds, and then frozen *in situ*. Determinations of lactic acid, inorganic phosphate, and the sum of inorganic and phosphocreatine-P were made, by the methods used previously. As in the previous work, the difference between the sum of inorganic and phosphocreatine-P of the resting and stimulated muscles was taken as the amount of hexosephosphate formed. Cori and Cori (1), using the 7-minute hydrolysis value, which includes in addition the labile P of the adenosine triphosphate, found that the values obtained by this procedure agree fairly well with those obtained by their method for the direct determination of hexosephosphate, which is much more involved. Of course, this procedure does not determine the actual quantity of hexosephosphate present, but for the present purpose it is only the difference between the resting and stimulated muscles that is significant.

The results obtained were those to be expected from the hypothesis. For the same duration of tetanus, the average amounts of lactic acid and of hexosephosphate formed are practically the same on the two diets. However, there is a marked difference in the amount of phosphocreatine *hydrolyzed*. On the "acid" diet this is two or three times as great as on the "alkaline" diet.

The amount of base liberated by the hydrolysis of phosphocreatine or by its conversion to hexosephosphate depends upon the pH of the medium. Fiske and Subbarow (4) have shown that the hydrolytic reaction yields the maximum amount of buffer at pH 5.6 to 5.8, where the base liberated amounts to 0.88 mole per mole of phosphocreatine hydrolyzed. By means of the Henderson-Hasselbalch equation it can be calculated that the conversion of one mole of phosphocreatine to hexosephosphate at pH 5.6 yields 0.68 mole of base. This value is obtained from the values for pK'_2 of 4.6 for phosphocreatine (4) and 6.12 for hexosephosphate (5).[1]

The calculations have been made for this particular pH value because Rous (14) has shown by means of intravital staining experiments that the pH of the muscle fiber, as distinguished from the tissue spaces, is at least as acid as pH 5.6. His experiments were performed on animals anesthetized with ether; Fiske and Subbarow have confirmed them on animals under amytal, which does not give rise to any acidosis, as ether may do. It is seen, then, that the buffering system of phosphocreatine hydrolysis has its maximum efficiency at the pH of the tissue in which it is present in greatest quantity. The pH of maximum liberation of base through formation of

[1] The equation showing this liberation of base and neutralization of lactic acid formed from glycogen is the following:

$$0.91\ C_4H_8O_2N_3PO_3K_2 + 0.09\ C_4H_8O_2N_3PO_3KH + 1.34/n\ (C_6H_{10}O_5)n + 1.34\ H_2O =$$
$$0.23\ C_6H_{11}O_6PO_3K_2 + 0.77\ C_6H_{11}O_6PO_3KH + 0.68\ C_3H_5O_3K + C_4H_9O_2N_3.$$

TABLE 1

Effect of change in diet on formation of lactic acid and hexosephosphate and hydrolysis of phosphocreatine in tetanus

Values expressed as milligrams percent of P and of lactic acid

RESTING MUSCLE			STIMULATED MUSCLE			DIFFERENCE			LACTIC ACID EQUIVALENT OF CHANGES IN PHOSPHOCREATINE AT pH 5.6	PER CENT OF LACTIC ACID NEUTRALIZED AT pH 5.6
Inorganic P	Inorganic plus Phosphocreatine P	Lactic Acid	Inorganic P	Inorganic plus Phosphocreatine-P	Lactic Acid	Phosphocreatine Hydrolysis	Hexosephosphate Formation	Lactic Acid Formation		
I. Duration of tetanus 5 seconds										
a. Rabbits on "alkaline" diet										
15	94	18	18	75	84	3	19	66	46	70
14	89	21	18	81	75	4	8	54	26	50
18	97	22	21	79	81	3	18	59	44	75
16	84	30	20	72	92	4	12	62	34	55
16	97	34	22	76	104	6	21	70	57	81
19	86	18	25	77	64	6	9	46	33	72
Average.........			....			4.3	14.5	59.5		67
b. Rabbits on "acid" diet										
18	101	35	27	86	86	9	15	51	53	104
16	97	25	39	80	108	23	17	83	92	111
17	104	23	23	86	74	6	18	51	51	100
12	88	26	23	74	69	11	14	43	56	130
21	101	23	30	86	83	9	15	60	53	88
19	105	13	29	87	77	10	18	64	61	95
Average.........			....			11.3	16.2	58.7		104.7
II. Duration of tetanus 15 seconds										
a. Rabbits on "alkaline" diet										
15	94	10	30	73	107	15	21	97	80	82
17	93	34	27	81	92	10	12	58	49	84
16	98	22	29	72	156	13	26	134	85	63
17	104	20	23	85	101	6	19	81	53	66
17	107	21	21	87	85	4	20	64	50	78
18	103	22	27	77	113	9	26	91	75	82
Average.........			....			9.5	20.7	87.5		76
b. Rabbits on "acid" diet										
14	94	27	28	70	112	14	24	85	83	98
17	112	20	25	88	92	8	24	72	68	94
20	108	12	45	85	129	25	23	117	109	93
17	105	21	24	87	78	7	18	57	54	95
16	106	23	31	86	93	15	20	70	78	111
17	91	33	37	72	131	20	19	94	88	94
Average.........			....			14.8	21.3	82.5		97.5

hexosephosphate is at a slightly more acid value, about 5.2, but the buffering efficiency at pH 5.6 is only slightly below the maximum.

Calculations have been made, on the basis of Rous' findings, of the quantity of lactic acid which would be neutralized by the base liberated in the two reactions of phosphocreatine. In the "alkaline" rabbits this amounts to from two-thirds (5-second tetanus) to three-fourths (15-second tetanus) of the quantity actually formed. In the "acid" rabbits, on the other hand, the calculated amount is equal to that actually formed, within the limits of experimental error. The conclusion is that the amount of phosphocreatine hydrolyzed during contraction is that which will furnish the amount of buffer necessary to prevent any change in pH of the muscle fiber. In the "alkaline" rabbits the amount of buffer liberated by the neutralization of alkali-protein is such that only a small amount of phosphocreatine need be hydrolyzed. In the "acid" rabbits this hydrolytic reaction must furnish a larger amount of buffer. The amount of base liberated by the formation of hexosephosphate is practically the same in the two series.

In order for the conclusion stated above to be valid, it is necessary to assume that there is little or no diffusion either of lactic acid or of the products of the hydrolysis of phosphocreatine out of the muscle *fiber* during the period of contraction. The diffusion of lactic acid from the muscle fiber into the tissue spaces is much slower than the diffusion from the tissue spaces, according to Eggleton, Eggleton, and Hill (2). Furthermore, it has been shown by Martin, Field, and Hall (10) that only a small amount of the lactic acid formed in a contracting muscle passes into the blood stream during the work period, and by Margaria, Edwards, and Dill (9) that little phosphate enters the blood during exercise. Naturally, it is quite difficult to prove that the diffusion out of the muscle fiber is slow, but these observations are all in harmony with such a concept.

In marked contrast to the different amounts of phosphocreatine hydrolyzed in the two series, the quantity which is converted to hexosephosphate is practically unaffected by the change in diet. In the Lundsgaard hypothesis no special significance is ascribed to this substance, so far as normal muscle is concerned. The prevailing view is that of Meyerhof (11), in which the substance is considered to be the stabilization product of a labile substance which is, in turn, the intermediate in the conversion of glycogen to lactic acid. In the first paper of this series, the significance of hexosephosphate formation was stated to be the same as that of lactic acid formation: both reactions furnish energy for work in the absence of oxygen. Lundsgaard's data on muscles poisoned with iodoacetic acid and the recent work of Kerly and Ronzoni (6) on the effect of pH on the carbohydrate changes in anaerobiosis, tend to support this interpretation. Under conditions in which one of these reactions is inhibited, the other appears as the chief product of the breakdown of glycogen, either in anaerobiosis or

in anaerobic work. The iodoacetate muscles are unable to form lactic acid, hence their capacity to perform work anaerobically is limited to that which can be done with the energy obtained from the formation of hexosephosphate—largely hexose-*di*phosphate in this case—from all the phosphocreatine present. Kerly and Ronzoni find that in 2½ hours' anaerobiosis of frog muscle at pH 6.0 there is a considerable accumulation of hexosephosphate and practically no lactic acid is formed; at pH 9.0 there is no hexosephosphate formed, but large amounts of lactic acid are produced. Changing the pH from 6.0 to 9.0 after the maximum amount of hexosephosphate formation has taken place does not cause any conversion of this substance to lactic acid. All these findings argue in favor of the view that the formation of lactic acid and of hexosephosphate have the same function, and against the theory that hexosephosphate is the intermediate in the formation of lactic acid, or a "stabilization product" of such an intermediate. They also tend to show that hexosephosphate is formed directly from phosphocreatine and not through the intermediate formation of phosphoric acid, as suggested by Lundsgaard (7).

There is apparently a marked difference in the relation between the hydrolysis of phosphocreatine and the formation of lactic acid in frog muscle from that which has been described here for mammalian muscle. Nachmansohn (13) and Lundsgaard (8) both find that in a tetanus the ratio of phosphoric acid formed to lactic acid formed is 2:1. If it is assumed that the pH of the frog muscle fiber is 5.6, the ratio should be 1.24:1. However, the "anaerobic resynthesis" of phosphocreatine after a tetanus amounts to some 30 per cent of that broken down during the tetanus, according to Nachmansohn. Allowing for this, the ratio of the net amount of phosphoric acid formed to lactic acid formed would become 1.4:1. Lundsgaard also finds that after three successive tetani, each of two seconds' duration, the ratio obtained is 1.2:1. These ratios are sufficiently close to the theoretical to permit the interpretation that in frog muscle also the function of phosphocreatine hydrolysis is to buffer against lactic acid. The excess hydrolysis can be regarded as an "overcorrection" of the increased acidity. The "anaerobic resynthesis" of the excess becomes only the restoration of pH equilibrium, and is not necessarily a fundamental part of the recovery process.

SUMMARY AND CONCLUSIONS

The data presented are in keeping with the following conclusions:

1. The hydrolysis of phosphocreatine in contracting mammalian muscle is dependent on the tissue alkali reserve.

2. The formation of hexosephosphate is independent of tissue alkali reserve and is parallel to the formation of lactic acid.

3. In animals with low tissue alkali reserve the amount of base liberated

by the hydrolysis of phosphocreatine and by the conversion of this substance to hexosephosphate is equivalent to the amount of lactic acid formed during contraction.

4. The function of the hydrolysis of phosphocreatine in contracting mammalian muscle is the preservation of a constant pH within the muscle fiber.

5. The conversion of phosphocreatine to hexosephosphate in contracting muscle serves both to furnish energy for work in the absence of oxygen and to buffer against part of the lactic acid formed concurrently.

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THE EFFECT OF INTRAVENOUS ADMINISTRATION OF THE PREGNANCY URINE FACTOR ON THE OVARIES OF RHESUS MONKEYS[1]

EARL T. ENGLE

From the Department of Anatomy, College of Physicians and Surgeons, Columbia University

Received for publication February 23, 1934

In a previous report evidence was presented which tended to show that the anterior-pituitary-like substance extracted from the urine of human pregnancy did not activate the ovarian follicle of the rhesus monkey (Engle, 1933).

Subcutaneous injections were given in relatively small doses, from 30 to 200 units daily, and for long periods of time, 20 to 30 days. In the present study are reported further attempts to obtain ovarian changes by modifying the method and the length of administration, and the amount of active principle given.[2,3]

Does P.U. damage the ovary? The changes in the ovarian follicle of the monkey which were previously reported are those of arrested development and involution in many follicles. It was desirable to learn if the ovary were damaged by the treatment or temporarily checked. The protocols of two animals are presented.

My 141—3/29/33—3850 grams. Slight anal redness. Rt. ovary removed, 135.0 grams. Day 7 after unilateral ovariectomy 1½ cc. Follutein (50 u.cc., subcu., 25 days, total 37 cc., 1950 units). On day 15 of treatment, pale gray, neutral, sex skin. On day following last PU treatment no inj. Then 2 cc. AP subcu. for 10 days. On day 5, red flush. Color and swelling progressed typically to day 10, when treatment was changed.

My 149—4/27/33—3150 grams. Left ovary removed; 137.0 grams. Two cubic centimeters Follutein (a pregnancy urine derivative), 100 units daily subcu., 24 days; 5 cc. daily, 12 days. Total 36 days, 108 cc., 10,800 units. No treatment after June 3. No change in sex skin during treatment. Animal rested, no treatment through summer. In August a normal development of sex skin, excellent color, good pudendal swelling, rugosities on thighs, followed by normal bleeding. A second period of activation occurred the first week in October.

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[1] Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

[2] The extracts of human pregnancy urine (Follutein, Squibb) was furnished through the coöperation of Dr. J. A. Morrell of E. R. Squibb & Sons.

[3] The dried sheep pituitaries were supplied through the courtesy of Dr. O. P. Kamm of Parke, Davis & Company.

The ovary of an animal treated with pregnancy urine is not permanently damaged, but again becomes functional. This resumption of function occurs slowly if treatment is stopped or will occur immediately if A.P. extracts are administered (see also My 172, below).

Intravenous injections of high dosages for short periods. It has appeared that extracts of pregnancy urine administered subcutaneously over varying periods of time, from 10 to 40 days, did not cause follicular activation or liberation of oestrin in the monkey.

In the present series, adult animals have been used. The extracts were administered by injections in the popliteal and posterior tibial veins to determine if the results differed from those obtained by subcutaneous injections. The dosages have been high, ranging from 3000 to 9900 units given over a period of three to five days. In many instances the extracts were diluted with sterile Ringer's solution before injection. When given very slowly, a daily intravenous injection of 10 cc. is accomplished without untoward effect. Occasionally thrombosis occurred when a glycerine extract was used.

None of these animals had been treated previously, and many had been observed through previous menstrual bleedings.

It was desired to start treatment in animals which already gave evidence, in the sex skin, of follicular activity. Such cases are presented in the detailed protocols below.

All comment on ovaries is based on a study of complete serial sections of both the control and experimental ovaries. A microphotograph of a single section is inadequate to portray the condition of the whole ovary. A difficulty in this study has been the lack of precise information regarding the normal ovary of the monkey in the menstrual cycle. As the number of normal ovaries available for study increases, the range of variability of morphological features is better understood. A description of the different experimentally modified ovaries is compared not only with the control ovary of the animal in question, but with the conditions found in a modest series of normal ovaries at various stages of the cycle.[4]

My 149—3250 grams (continuation of protocol recorded above). After normal bleeding 14 days previous, excellent genital swelling and redness. At peak of this condition 2 cc. of PU derivative (Follutein) 800 R.U. daily intravenously for 4 days, total 3200 R.U. Rt. ovary, 8.6 x 6.4 x 4.6; 137 mgm.

All larger follicles are in advanced atresia. The granulosa is gone in all save a few follicles. Ova free in antrum. Theca thickened, with luteoid

[4] All measurements were made with a Vernier caliper. Those made of an ovary which had been removed are reasonably accurate, the length in three axes being recorded. Measurements of the intact ovary, particularly the length, are not so accurate, distortion due to manipulation being unavoidable.

cells. Lumina of many follicles had been obliterated by the process of luteoid involution. Small follicles were unchanged. No typical corpora lutea were present.

My 165 ♀—4650 grams. Marked reddening and rugosities. Rt. ovary removed, 8.6 x 4.7 x 4.2; 144.1 mgm. Left ovary, 10.2 x 5.4 x 4.0 intact. Left ovary much larger. No observable corpora. A pregnancy urine product, Follutein, containing 1250 RU per cc. given intravenously.

Day 1 Rt. ovary removed
Day 4 1½ cc. 2 times, 3750 RU. i.v.
Day 5 2½ cc. once, 3075 RU. i.v.
Day 6 2½ cc. once, 3075 RU. i.v.
Day 7 ovariectomy 9900 RU.

Left ovary, 8.6 x 5.0 x 4.0; 115.0 mgm. Profuse uterine bleeding from day 5.

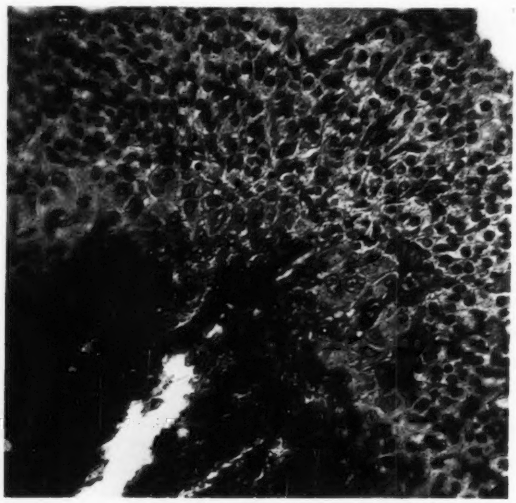

Fig. 1. Section of follicle wall of the left ovary of My 165. This is one of several hemorrhagic follicles in the treated ovary. Luteoid changes are apparent in the granulosa. No greater changes toward a luteinized condition were obtained in this series with the pregnancy urine factor only.

This preparation, in glycerine solution, caused extensive thromboses of the veins, and prevented further intravenous treatment. The ovarian response was more nearly toward lutein development than any of this series. The granulosa was not so completely destroyed as in the previous case (My 149) and the theca in the larger follicles showed definite lutein cells. The granulosa cells also showed decided change in the nucleo-cytoplasmic ratio but there were only a few typical lutein cells (fig. 1). Numerous hem-

orrhagic follicles were present. Extensive atresia of all small follicles was evident. The epithelium of the uterine tube showed slight secretory change toward the progestational condition, but was not a true post-ovulatory type.

My 170—4450 grams. Intensive red of sex skin, some rugae; left ovary removed, 9.2 x 7.8 x 5.0; 196.8 mgm. Rt. ovary, larger in mass, no corpora, 9.0 x 7.4 x 7.4, left intact. Follutein, given intravenously on day following operation.

Day 1—3 cc.	=	3750 RU. i.v.
Day 2—1½ cc.	=	1875 RU. i.v.
Day 3—1½ cc.	=	1875 RU. i.v.
Total.........		6500 RU.

Day 4—Rt. ovary removed, 7.9 x 7.4 x 4.6; 202.6 mgm. External uterine bleeding on fourth day after 1st injection, fifth day after removal of 1st ovary.

Numerous large follicles are present in both ovaries. In the treated ovary are still a few apparently normal follicles with a granulosa which is not abnormal. Numerous other follicles of the same size are denuded of granulosa. Little change of a luteal nature. Large numbers of small hyalinized follicles with cytolized ova. This animal shows less damage and change in the large ovarian follicles than any of the animals of this series.

Three additional animals are reported in which treatment began after the peak of oestral activity, as estimated by the condition of the sex skin, had been reached, or definitely passed. As in those reported above, the treatments consisted of intravenous injections of high dosages for short periods.

My 164—4500 grams. Just past peak of color and swelling. Rt. ovary removed, 8.4 x 5.4 x 4.0; 128.1 mgm. Left ovary intact, 9.9 x 5.8 x 5.0. No observable corpora lutea in either ovary. PU derivative (Follutein, 250 u.cc.).

Day 1—3 cc.	=	750 RU. i.v.
Day 2—3 cc.	=	750 RU. i.v.
Day 3—1½ cc. × 2	=	750 RU. i.v.
Day 4—1½ cc. × 2	=	750 RU. i.v.
Total...........		3000 RU.

Day 5—Left ovary removed; 8.1 x 5.2 x 4.4; 135.1 mgm.

There is not a normal follicle in the large or medium sized groups in the treated ovary. Atretic changes profound, with less luteoid modification than in My 165. Complete hyalinization of scores of medium sized follicles.

My 169—5000 grams multipara. Sex skin beginning to pale. Taken as soon as peak of oestral activity had passed. Left ovary removed, 7.8 x 4.3 x 4.0; 89.9 mgm. Rt. ovary larger, 10.0 x 4.2 x 4.8, left intact. Follutein, 1250 RU per cc. given intravenously, day after ovariectomy.

Day 1—2½ cc. = 3125 RU. i.v.
Day 2— 1 cc. = 1250 RU. i.v.
Day 3— 1 cc. = 1250 RU. i.v.
Total........ 5650 RU.
Day 4 none
Day 5 none
Day 6 ovariectomy.

Rt. ovary, 7.8 x 4.6 x 3.7; 99.2 mgm. Day 4 color entirely faded, rugae smoothed out, vaginal bleeding.

The experiment on this animal started after the oestral activity had decidedly passed the peak. The control ovary shows one large atretic follicle, but no corpora. There were more of the small atretic, sclerosed follicles than is usual in a normal ovary. Two days elapsed between the last treatment and the removal of the experimental ovary.

Very few normal follicles are present; many have undergone atresia with cytolysis of the entire granulosa; others with varying degrees of thecal hypertrophy.

Intravenous administration of small dosages. In none of the monkeys studied, whether treated with moderate dosages (8000 units) in 22 days, or treated with large doses (over 800 units per day) for a short period showed any indication of follicular activity. Due to the inhibitory or regressive nature of the ovarian response, it was feared that all the doses given might be too massive to induce follicular activity and oestrus production. It therefore seemed worth while to give small dosages to some animals. Two animals of an age and size known to respond to the follicle activator of the anterior pituitary gland were used. The sex skin of both animals was neutral, and it is judged that neither previously had shown activation of the sex skin.

My 173—2850 grams. Both ovaries intact; sex skin neutral. Treated with Antuitrin S, R 095402F, an especially good fraction from pregnancy urine. One-tenth cubic centimeter (10 RU.) per day intravenously for 12 days. There was no change in sex skin at any time.

My 172—3400 grams. Sex skin neutral, with a tinge of redness between ischial tuberosities. Given same dosage of the same preparation, $\frac{1}{10}$ cc. i.v. for 12 days. As in the other animal there was no change indicating the slightest ovarian activation.

As a further step, to ensure the responsivity of this type of animal, My 172 was treated subsequently with pyridine extract of the AP. An old preparation assaying less than 10 units per cc. was given subcutaneously for 8 days. On the 6th day a slight redness was noted and on the 7th and 8th days a moderate redness was accompanied by vulvar and anal swelling. The response was not as good as is obtained with the usual larger dosage, but was sufficient to show that the animal would respond to an adequate activator.

Discussion. The anterior-pituitary-like principle of human pregnancy urine does not cause follicular activation and oestrin production

in the rhesus monkey when given intravenously in very small dosages (10 RU. pro diem) or massive dosages (800 RU. to 3750 RU. pro diem). Several adult animals of the present series were normally in the oestrin phase of the cycle, with daily evidences of increase in the color intensity and rugosity of the sex skin. Upon intravenous administration of massive doses of the pregnancy urine factor, the color began to fade. This is a pronounced and unfailing feature of treatment of adult or sub-adult animals, which points to the checking of oestrin production by the ovarian follicle. Uterine bleeding of oestrin privea[5] occurred in most instances. Oestrin production was not stimulated to compensate for the removal of one ovary.

None of the animals treated with the pregnancy urine principle alone have caused the production of full-sized corpora lutea in the ovary. It is suspected that there may be a time factor here, and that the injections should be continued for a longer time, and that the ovary should be taken after the height of follicular proliferation. Such luteal changes as have occurred have been mainly in the theca interna.

The granulosa is in many instances completely cytolized, and scores of the small hyalinized or sclerosed bodies previously mentioned occur. They appear to be the terminal phases of the involution of atretic follicles, and as mentioned in an earlier paper, occur in all normal ovaries. In these experimental ovaries the condition is exaggerated as to the extent and number of follicles showing this atretic condition. The ovary in every instance was smaller after initiation of the experiment. The technique of measuring the removed ovary permits a more accurate determination than is obtained on an intact organ. It is granted that in these animals measurement on an abdominal ovary is only an approximation. Both these measurements and the subjective visual estimate of mass leads one to think that there has been actual shrinkage in the ovary as a result of treatment. It seems hardly necessary to state that traumatic manipulations of the ovary at operation are slight, and especial care is taken to avoid damage to the blood supply.

The recent report of Geist (1933) indicates that the same type of arrest of follicular development occurs in the human ovary. In Geist's cases, as in the monkey, the hemorrhagic follicle is a common if not constant result of the treatment. Such a hemorrhagic follicle has been seen once in a small follicle in a single normal monkey ovary, and may occur in animals treated intravenously with an A.P. fraction.

SUMMARY

1. The anterior-pituitary-like principle of human pregnancy urine not only fails to cause follicular activation, but definitely inhibits the produc-

[5] "Bleeding of oestrin privea" is the Allen phenomenon of uterine bleeding as a result of the deprivation of oestrin supply of the monkey.

tion of oestrin, as judged by loss of color of sex skin and oestrin privea uterine bleeding.

2. The previous observations on this point were extended by experiments on nine rhesus monkeys. In 5 animals intravenous dosages ranging from 3000 rat units in four days to 9900 units in four days.

3. The inhibition of oestrin production is accompanied by hyalinzation of smaller follicles, extensive cytolysis of the granulosa, and thecal thickening. These morphological changes are interpreted as being various phases in follicular atresia.

4. The ovary of the monkey is not damaged, as in 1 animal it was reactivated within the normal time by an active fraction of the anterior pituitary: in another, it entered a normal cycle after cessation of treatment.

5. The intravenous administration of this active principle in adult monkeys, either in massive doses or in very small doses fails to cause oestrin production.

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CHLORIDE, CARBOHYDRATE AND WATER METABOLISM IN ADRENAL INSUFFICIENCY AND OTHER CONDITIONS[1]

HERBERT SILVETTE

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication February 10, 1934

Reductions in blood and plasma chloride concentration after adrenal removal have been noted by various investigators (Lucas, 1926; Rogoff and Stewart, 1926; Baumann and Kurland, 1927; Ohguri, 1931). Accompanying the fall in plasma chlorides is a dehydration of the blood (the above authors; Swingle and Eisenmann, 1927; Silvette, 1933), a diminution in blood volume (Wyman and tum Suden, 1930; Swingle *et al.*, 1933), a marked hydration of liver and muscle tissue (Silvette and Britton, 1933), a drop in serum sodium (Baumann and Kurland, 1927; Loeb, 1932, 1933; Harrop *et al.*, 1933), and a reduction in urine volume, although the urinary output of chlorides and sodium is increased (Silvette and Britton, 1933; Loeb, 1933; Rubin and Krick, 1933). The administration of various saline solutions has been shown to lengthen the life-span of adrenalectomized animals (Marine and Baumann, 1927; Corey, 1927; Britton, 1930), while the ingestion of sodium chloride by Addisonian patients in crisis is said to relieve the condition and thereafter to maintain the subject in good health (Loeb; Harrop *et al.*).

These considerations lead to the belief that the adrenal cortex is in some manner related to salt and water metabolism. It is interesting to note that the injection of sodium chloride increases the blood sugar, the depletion of which after adrenalectomy has already been emphasized (Britton and Silvette, 1931, 1932, 1933, 1934). It is significant also that the two tissues which show increased hydration after adrenal removal are precisely those which show profound reductions in glycogen content.

Methods. Serum and tissue chloride analyses were performed on fresh material by Van Slyke and Sendroy's method (Peters and Van Slyke, 1932). Serum sugar was determined by the ferricyanide technique of Folin and Malmros (1929), glycogen by a modification of Pflüger's method previously described (Silvette and Britton, 1932), and moisture content by drying the tissue to constant weight in an electric oven maintained at 104°C.

[1] The data in this paper are taken from a dissertation presented to the Graduate Faculty of the University of Virginia in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Results. *Normal and fasting animals.* The chloride and water content of blood serum, liver and muscle of normal adult cats is given in table 1. Average values obtained from a group of mature male white rats are presented in table 6. The results of the analyses compare favorably with others on similar and different species quoted in the literature (Katz, 1896; Cameron and Walton, 1928).

A series of normal cats was fasted for different periods, as noted in table 2, and the tissues and serum then analyzed. Both liver and muscle

TABLE 1

Chloride and water content of serum, liver and muscle of 12 normal cats, fasted 18 hours

	WATER			CHLORIDES			SERUM SUGAR
	Serum	Muscle	Liver	Serum	Muscle	Liver	
	per cent	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
Maximum	93.63	77.2	73.8	441	54	114	138
Minimum	91.15	74.5	62.4	411	38	85	64
Average	91.97	75.7	69.5	429	48	102	88

TABLE 2

Effect of moderately prolonged starvation on the water, chloride and carbohydrate levels of cat tissues

	SURVIVAL	BODY WEIGHT LOST	WATER			CHLORIDES			SERUM SUGAR	GLYCOGEN	
			Serum	Muscle	Liver	Serum	Muscle	Liver		Muscle	Liver
	days	*per cent*	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
Maximum	12	27.2	92.34	77.6	74.1	452	52	95	93	0.29	0.44
Minimum	10	13.7	91.40	74.9	68.3	420	30	77	67	0.17	0.26
Average	11	19.7	91.81	76.0	71.2	433	40	85	81	0.23	0.33
No. animals	7	7	7	7	6	7	7	7	7	7	7

gained water and lost chlorides and glycogen, while the serum remained normal in composition. Similar results were obtained in a series of white rats fasted for a comparable length of time (table 6).

Adrenalectomy. Analyses of blood glucose and liver and muscle glycogen content performed on short and long-surviving groups of adrenalectomized cats give evidence of a progressive decline in carbohydrate reserves (Britton and Silvette, 1932). A similar division into two categories with respect to water and chloride balance is apparent from a consideration of

table 3. The experiments indicate that there occurs an increase in muscle water accompanied by a decrease in serum, liver and muscle chlorides after adrenalectomy. These changes are progressive and related to the period of survival after operation.

The increased hepatic chloride levels observed in cats dying one to three days after operation are more apparent than real. Simple inspection of the liver of such animals reveals an organ turgid with blood which exudes from the cut surface more rapidly and in greater amount than is the case in unoperated controls. The increased hepatic water content of adrenalectomized animals appears to be due to an abnormal distention of the hepatic vessels and sinuses with blood. Calculations based on this assumption explain the increase in liver chlorides in short-lived adrenalectomized cats:

TABLE 3

Tissue chlorides and water and serum sugar following adrenalectomy in cats

CAT NO.	SURVIVAL	CONDITION WHEN KILLED	WATER			CHLORIDES			SERUM SUGAR
			Serum	Muscle	Liver	Serum	Muscle	Liver	
A. Short-surviving animals									
	days		*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
1	3	In convulsions	92.10	76.0	70.0	404	49	130	40
2	3	Weak	91.27	75.6	73.3	424	36	121	59
3	2	Weak	91.57	77.0	74.9	392	48	135	68
4	1	Weak	91.84	75.4	73.0	431	36	122	50
5	1	Weak	92.80	76.1	73.1	403	31	101	84
6	1	Weak	92.07	76.5	73.7	406	34	108	75
7	2	Prostrated	92.34	76.0	72.2	406	46	143	64
8	2	Prostrated	91.93	76.8	72.6	435	54	102	72
9	2	Weak	92.58	76.4	73.8	441	52	120	80
10	3	Moribund	90.18	75.4	73.1	426	52	140	38
11	2	Moribund	90.08	76.1	76.6	425	42	120	79
B. Long-surviving animals									
12	6	Prostrated	92.18	77.8	73.7	392	38	89	77
13	4	Prostrated	91.08	78.6	74.5	439	36	93	80
14	5	Moribund	91.30	78.3	73.6	409	26	85	72
15	12	In convulsions	92.06	79.5	68.2	401	40	90	71
16	12	Very weak	91.20	77.7	73.0	382	28	86	70
17	6	Weak	91.50	76.3	73.5	408	41	97	85
18	8	In convulsions	91.06	77.2	74.1	385	41	82	76
19	5	Very weak	91.80	77.5	72.8	396	31	88	70
20	5	Weak	91.38	78.2	71.7	405	30	79	80

The liver weight of an adrenalectomized cat is equal to about 2.6 per cent of its body weight. The liver weight of a one-kilo adrenalless cat will therefore contain 26

× 0.733[2] or 19.1 grams of water. Twenty-six grams of normal hepatic tissue contain 26 × 0.695[3] or 18.1 grams of water. Thus, the liver of an adrenalectomized animal holds 1.0 gram of water more than an equal weight of normal tissue. This amount of water is contained in (approximately) 1.2 cc.[4] of blood and is equal to about 4.2 mgm. of chlorides.[5] But the 26-gram liver in adrenal insufficiency holds 31.7 mgm. of chlorides, i.e., 5.2 mgm. more than a normal liver of the same size.[6] Thus, the increased hepatic chloride after adrenal removal is almost equalled by the amount of chloride contained in the extra blood held in the congested liver.

It is therefore probable that in adrenal insufficiency the liver chlorides pursue a progressive decline similar to that observed in blood serum and muscle. The kidney excretes chlorides in abnormal quantities after adrenal removal, although the fluid output is concomitantly reduced (Silvette and Britton, 1933). The accompanying loss of tissue chlorides therefore appears referable to the enhanced renal excretion of the substance. With respect to water balance the conditions are reversed. The oliguria which is observed is attended by an increase of water in the tissues, while the concentration in the muscle (the main fluid depot of the body) progressively increases with the survival time.

The liver glycogen of adrenalectomized cats is depleted to a far greater extent than that of long-fasted controls. The glycogen values are reduced within 48 hours after adrenalectomy to 50 per cent of those observed in cases of prolonged fasting up to 12 days. The diminution of hepatic chlorides and the increase in liver water occur to the same degree in both starvation and adrenal insufficiency. The carbohydrate, water and chloride levels noted in starved cats were not prejudicial to normal function. The chloride and water balance in adrenalectomized animals cannot therefore be considered incompatible with normal life.

Rats dying after adrenal ablation with typical symptoms show increased liver and muscle water concentrations. Serum and liver chlorides are considerably reduced, but muscle chlorides are definitely increased (table 6). Comparison of the results with those obtained from a group of fasted animals reveals an essential similarity between the two (adrenalectomized and fasted) conditions.

[2] Liver water after 1 to 3 days of adrenal insufficiency is equal to 0.733 gram per gram of tissue (table 6).

[3] Normal hepatic tissue contains 0.695 gram water per gram (table 6).

[4] Blood of adrenalectomized animals is only 80.5 per cent water (Silvette and Britton, 1933). One cubic centimeter of water is approximately equivalent to 1.0/0.805 or 1.2 cc., correcting for the specific gravity of the blood.

[5] Blood of adrenalectomized cats contains about 3.4 mgm. chlorides per cubic centimeter (Baumann and Kurland, 1927).

[6] Liver of short-surviving animals contains 1.22 mgm. Cl per gram (table 6); normal hepatic tissue contains only 1.02 mgm. (table 6). Therefore, (26 × 1.22) − (26 × 1.02) or 5.2 mgm. Cl is the absolute increase in a 26 gram liver of a cat following adrenal removal.

Salt-feeding of normal and adrenalectomized animals. A series of cats was adrenalectomized and a similar number of normal animals of approximately the same body weight taken as controls. The animals in both series were each given *per os* a capsule containing two grams of sodium chloride in the morning and another in the evening of each day of survival. The standard laboratory diet was otherwise followed and water allowed *ad lib.* It was observed that the salt-fed adrenalless animals succumbed rather more quickly to the effects of the operation than untreated adrenalectomized cats. Each operated animal which was sacrificed when showing terminal

TABLE 4

Effects of salt-feeding on the chloride content of tissues of normal and adrenalectomized cats

CAT NO.	SURVIVAL	CONDITION WHEN KILLED	WATER			CHLORIDES			SERUM SUGAR	GLYCOGEN	
			Serum	Muscle	Liver	Serum	Muscle	Liver		Muscle	Liver
A. Normal animals											
	days		*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
21	2	Normal	92.45	75.2	66.6	445	39	66	89		1.31
22	3	Normal	92.82	75.9	70.8	462	40	82	79	0.55	1.22
23	2	Normal	92.11	75.9	68.6	435	35	70	78	0.51	1.78
24	2	Normal	92.55	76.5	62.7	444	53	69	103		
25	3	Normal	93.48	75.8	71.6	454	45	87	81	0.58	1.28
B. Adrenalectomized animals											
26	2	Prostrated	91.73	77.2		444	62	79	126	0.58	0.24
27	3	Prostrated	92.84	77.1	75.0	419	44	79	134	0.36	0.24
28	2	Moribund		75.9	73.3	456	45	108	37	0.60	0.32
29	2	In convulsions	93.29	77.4	74.9	470	46	92	85	0.52	0.25
30	3	Moribund	90.60	76.1	72.4	418	44	102	50	0.44	0.20

symptoms of insufficiency was compared with a normal, salt-fed control sacrificed at the same time. Five pairs of animals were thus examined. The results of the experiments are given in table 4, A and B.

The augmented chloride concentrations in the tissues examined indicate that adrenalectomized animals absorb ingested salt more slowly and retain it longer than the normal organism. Distribution of the absorbed chloride in the various tissues takes place normally, however, as judged by the parallel increases in serum, muscle and liver chlorides over the levels observed in normal salt-fed cats.

Carbohydrate metabolism and chloride and water balance: Pancreatectomy. Table 5A presents the water, chloride and carbohydrate balance following

removal of the pancreas in a series of cats. Serum sugar is greatly increased concomitantly with a profound serum chloride depletion in certain cases. On the average, liver and muscle chloride were reduced and the water increased, but wide variations in the data derived from different animals preclude a valid interpretation of the results.

TABLE 5

Observations on tissue water and chlorides under various experimental conditions involving carbohydrate metabolism

	WATER			CHLORIDES			SERUM SUGAR	GLYCOGEN	
	Serum	Muscle	Liver	Serum	Muscle	Liver		Muscle	Liver
A. Pancreatectomy (7 animals used)*									
	per cent	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
Maximum....	91.70	76.1	75.2	408	43	114	527	0.84	0.25
Minimum....	90.00	73.6	66.7	252	23	58	303	0.29	0.15
Average....	91.01	74.8	71.9	335	34	80	420	0.44	0.19
B. Glucose injection (4 animals)†									
Maximum....	92.05	76.6	74.7	410	42	101	263		5.23
Minimum....	91.35	75.2	70.0	396	29	81	134		2.16
Average....	91.78	76.0	71.9	402	35	89	184		3.68
C. Adrenalin injection (4 animals)‡									
Maximum....	92.20	77.0	74.2	443	50	106	308	0.40	0.54
Minimum....	91.26	74.8	72.1	419	39	74	165	0.23	0.42
Average....	91.90	75.8	72.8	430	42	86	235	0.32	0.48
D. Insulin injection (8 animals)§									
Maximum....	93.12	77.4	75.4	464	65	116	74	0.34	1.94
Minimum....	90.60	72.7	69.7	435	31	71	38	0.21	0.40
Average....	91.95	76.2	72.4	453	44	93	60	0.26	0.86

* Survival, from 2–5 (average 3) days.

† Seven hourly intraperitoneal injections of 10 cc. per kilo 5 per cent glucose.

‡ One cubic centimeter 1:1000 adrenalin per kilo body weight, injected subcutaneously.

§ Six units insulin per kilo body weight, injected subcutaneously.

Glucose injection. Normal cats given seven (hourly) injections of glucose were sacrificed one hour after the last injection. The quantity of sugar administered at any one time was not sufficient to overwhelm the glycogenic abilities of the organism, and the serum sugar did not rise much above the renal threshold value. Analysis showed large amounts of liver glycogen, a water balance approximating the normal, and a depletion of serum

and tissue chlorides (table 5B). This depletion may be explained by the diuresis brought about by the injection of fluid, and by the observed secretion into the peritoneal cavity of large amounts of chloride, apparently to replace the glucose absorbed from the injected solution.

TABLE 6

Effect of different experimental procedures on water, chloride and carbohydrate levels of serum, muscle and liver

NO. ANIMALS	SPECIES	EXPERIMENTAL CONDITION	SURVIVAL	WATER			CHLORIDES			SERUM SUGAR	GLYCOGEN	
				Serum	Muscle	Liver	Serum	Muscle	Liver		Muscle	Liver
			days	*per cent*	*per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
12	Cat	Normal		91.97	75.7	69.5	429	48	102	88	0.43‡	1.22‡
7	Cat	Fasting	11	91.81	76.0	71.2	433	40	85	81	0.23	0.33
11	Cat	Adrex*	2	91.71	76.1	73.3	418	43	122	64	0.35§	0.19§
9	Cat	Adrex†	7	91.50	77.9	72.8	402	35	88	76	0.21§	0.07§
5	Cat	Normal, NaCl-fed		92.68	75.9	68.0	448	42	75	86	0.55	1.40
5	Cat	Adrex, NaCl-fed	2.4	92.11	76.7	73.9	442	48	92	86	0.50	0.25
7	Cat	Pancreatectomy	3	91.01	74.8	71.9	335	34	80	420	0.44	0.19
4	Cat	Glucose injection		91.78	76.0	71.9	402	35	89	184		3.68
4	Cat	Adrenalin injection		91.90	75.8	72.8	430	42	86	235	0.32	0.48
8	Cat	Insulin injection		91.95	76.2	72.4	453	44	93	60	0.26	0.86
7	Rat	Normal			72.6	69.6	408	50	95	130	0.47‖	1.05‖
5	Rat	Fasting	7.2		77.2	74.6	407	68	81	90	0.20	0.52
10	Rat	Adrenalectomized	17.5		77.3	73.8	367	56	82	73	0.29	0.17

* Adrex = adrenalectomized, short-survival.
† Adrex = adrenalectomized, long-survival.
‡ From table 8, Silvette and Britton, 1932.
§ From table 6, Britton and Silvette, 1932.
‖ From table 5, Britton and Silvette, 1934.

Adrenalin and insulin administration. The hyperglycemic influence of adrenalin was utilized in an attempt to increase the blood sugar without notably increasing the fluid intake of the body. Relatively large doses of adrenalin were injected subcutaneously into normal 18-hour fasted cats. The serum sugar was maintained above 200 mgm. per cent for several hours but no significant changes in water or chloride balance were observed (table 5C).

Normal, fasted animals were maintained under the influence of insulin at the point of incipient convulsions for two or three hours and then sacrificed (i.e., from five to seven hours after the injection). Serum sugar was low, and serum chlorides slightly though definitely increased. Hepatic and muscle chlorides were within the normal limits (table 5D).

Discussion. Herrick in 1924 first drew attention to a so-called reciprocal relation between sugar and chlorides in the blood. Later work (Ni, 1926) confirmed his observations and explained them on the basis of osmotic compensation. An increase in blood sugar demanded a compensatory decrease in chlorides in order to maintain the osmotic equilibrium of the blood.

The experiments reported herein have some bearing on this contention. The high serum sugar characteristic of pancreatectomy is found to be accompanied by an extremely low serum chloride level only when the glucose concentration is around 400 mgm. per cent. Contrastingly, the hyperglycemia following adrenalin injection is not accompanied by a low serum chloride, and the hypoglycemia of adrenal insufficiency is correlated with a low chloride level in the blood. Furthermore, the injection of sodium chloride or calcium chloride increases blood sugar (Rewbridge and Andrews, 1930; Silvette, unpublished observations). A true reciprocal relationship between serum sugar and chlorides does not, therefore, appear to exist. When the blood sugar is increased to extremely high levels, as after pancreatectomy or in diabetes, an osmotic compensation undoubtedly does occur. But when the sugar percentage is only moderately increased, or moderately or severely decreased, the osmotic pressure of the blood is is not sufficiently changed—due apparently to the ready diffusibility of glucose through the cell membranes—to demand a compensatory shift in the electrolytes of the blood.

Recent work on the correlation between glycogen and water content of the mammalian liver demonstrates that the degree of hydration of the tissue is not dependent on the carbohydrate level (Bridge and Bridges, 1931; Holmquist, 1932; Puckett and Wiley, 1932). Conditions which either increase or decrease liver glycogen are found to bring about hydration; indeed an increase in liver water is one of the commonest changes noted after various experimental procedures, e.g., nephrectomy, pancreatectomy, starvation, glucose, adrenalin and insulin injection. The ready changes in liver volume which are brought about by numerous chemical and nervous forms of stimulation render invalid any specific interpretations based on hepatic water shifts.

Several observers have recently stressed the increases in muscle water which occur after adrenal removal, and have explained the findings on the basis of increased endothelial permeability (Winter and Hartman, 1933; see also Gradinescu, 1913; Viale and Bruno, 1927; Monauni, 1929). Monauni

explained this phenomenon in muscle on apparently more logical grounds, to wit, an increased avidity of the tissue for water. "It is generally believed that the permeability of tissues to water does not change significantly with functional activities, but that the permeability to substances exerting osmotic pressure changes, and by this means the movement of water is modified" (Adolph, 1933). The decrease in muscle chlorides, occurring as it does progressively and in inverse proportion to the muscle water increase, is probably of importance in regulating osmotic balance in the adrenalectomized organism.

It would be more proper, perhaps, to speak of sodium chloride rather than of chloride alone, for the sodium ion in adrenal insufficiency pursues the same course as the chlorion. Sodium and chlorides are excreted abnormally by the kidneys, and are both depleted in the blood plasma; sodium chloride relieves the crisis in Addison's disease and increases the survival period of adrenalless animals. The fact that sodium chloride is excreted while water is retained renders it highly probable that the renal mechanism is so adjusted in the absence of the cortical hormone that the osmotic pressure of the blood and tissues is held at some optimal level.

The conditions underlying water retention and salt excretion are to be sought after in the tissues themselves. The evidence at hand indicates that the glucose and glycogen deficiency of the adrenalectomized animal constitutes the underlying factor. It is possible to reproduce the water and salt shifts which are observed in adrenal insufficiency in numerous other experimental conditions; reëstablishment of normal water balance after adrenalectomy is furthermore readily effected by non-hormonal means, e.g., simple saline injections (Britton and Silvette, 1934). The only procedure which has so far been observed to increase adequately the depleted hepatic glycogen reserves characteristic of complete adrenal insufficiency is the injection of the specific hormone of the adrenal cortex.

SUMMARY

The water content of the muscle in cats progressively increases after adrenal removal while the chloride concentration in the tissue falls. Hepatic water and chlorides follow the same course, and serum water and chlorides slowly though steadily fall.

There appears to be a marked similarity between water and chloride metabolism during starvation and in adrenal insufficiency. A significant difference is the maintenance of a normal serum chloride level by the fasting animal, whereas the chloride concentration in adrenalectomized animals showing more or less severe symptoms of insufficiency is characteristically low.

No correlation has been found between tissue chlorides and carbohydrates with one exception, i.e., after pancreatectomy the serum glucose and chloride levels reciprocally vary.

Many experimental procedures reproduce the condition of muscle and liver hydration noted after adrenal removal, and it is possible also to induce and maintain low chloride and high water levels in the tissues without prejudice to the well-being of the animal.

The changes in water and chloride balance noted after adrenalectomy do not appear to be specifically related to the absence of the cortical hormone, but probably result indirectly from some underlying and more fundamental condition which is produced by lack of the hormone.

Grateful acknowledgment is made of the kind encouragement and helpful advice of Prof. S. W. Britton throughout these studies.

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THE HEMOPOIETIC EFFECT OF COBALT AND COBALT-MANGANESE COMPOUNDS IN RABBITS

WILLIAM KLEINBERG

From Washington Square College, New York University

Received for publication March 10, 1934

In a study of the toxicity of various metals, Waltner and Waltner (1929) found that polycythemia is produced by the addition of from 0.5 per cent to 2 per cent pulverised metallic cobalt to the diet of normal rats or when from 0.01 to 0.1 gram of cobalt chloride or cobalt nitrate is injected subcutaneously. An increase in red cells and hemoglobin as a result of the oral administration of cobalt to dogs was observed by Mascherpa (1930), who corroborated the alleged hemopoietic effect of cobalt by finding, on gross examination, an abnormally red and hyperplastic bone marrow. Myers, Beard, and Barnes (1931–1932) have reported that 1 per cent of cobalt added to the mixed diet of rats as cobalt chloride, produces a polycythemia. Orten, Underhill, and Lewis (1931–1932) found that 0.5 mgm. of cobalt, as cobalt chloride or cobalt sulphate, produces a polycythemia in rats when fed with copper as a supplement to a milk-iron diet. In a later paper (1932a) they found that an increase of blood volume accompanies the erythremia, and also that if manganese, as manganese chloride, is added to the diet containing cobalt, the red cell and hemoglobin increases become more regular, and the toxicity of the cobalt is diminished (1932b).

The hemopoietic effect of cobalt has thus been studied under different conditions, in different chemical combinations as well as uncombined, and in different mammals, and there is an increase of erythrocytes, a corresponding increase of hemoglobin, an increase of blood volume, and gross hyperplasia of the bone marrow. This paper will show that the administration of cobalt and cobalt-manganese compounds produces other changes which are recognized as signs of bone marrow activity, particularly an increase of reticulocytes.

Method. Rabbits of both sexes, all colors, and ranging in weight from four and a half to six pounds, were used for the experiments. Their diet before, during, and after the experimental period was a mixed one of cabbage, carrots, hay, and oats.

Red cell and reticulocyte counts were made only from samples of blood

taken by cardiac puncture.[1] White cell, differential, and polynuclear counts were made either from the cardiac blood sample or from one taken from a marginal ear vein. The variation in the white cell count between cardiac and peripheral blood, if any, is negligible since large spontaneous changes occur.

Red cell counts were checked in each sample by making counts on both sides of a double hemocytometer. White cell counts were made on one side of the hemocytometer. All counting and diluting equipment was certified by the Bureau of Standards. The reticulocytes were stained with brilliant cresyl blue and counted in wet mounts according to the method of Ramsey and Warren (1932). These counts were made in duplicate, one thousand red cells being counted on each of two slide preparations and the reticulocytes expressed as a percentage of the total red cells.

Cobalt, as cobalt chloride and cobalt nitrate, and manganese chloride were used. All chemicals were of the purest grade obtainable.

RESULTS. A. *Single injections.* Single, subcutaneous injections of cobalt chloride, of such an amount as to supply 10, 20, 40, 60, 120, and 240 mgm. of Co, were given to six littermate rabbits, ranging in weight from four and half to five and a quarter pounds. This was done in order to determine both the toxicity and the hemopoietic effectiveness of single doses. Those animals which had received the three greatest doses (i.e., 60, 120, and 240 mgm. of Co), died within twenty-four hours after the injection. The animals which had received 10 and 20 mgm. showed no symptoms, while the animal injected with 40 mgm. was slightly lethargic for a day.

Daily reticulocyte counts and total red cell counts in the animals remaining alive showed no significant rise over a period of two weeks after the injection. A left handed deflection of the polynuclear count and a transient lymphocytosis was observed and will be discussed below.

B. *Multiple injections.* In order to simulate by injections the conditions in which polycythemia is developed in rats and dogs by continuous feeding, 10 mgm. of cobalt, as cobalt chloride, were given daily to six matched rabbits. A very marked hemopoiesis resulted as evidenced especially by the striking rise in reticulocytes to 13 per cent to 18 per cent in about sixteen days. The results for two representative animals are shown in figures 1A and 1B. A slight drop in the red cell count usually occurs for the first few days and is followed by fluctuation about the normal mean of 6,500,000. There is no observable relation between the reticulocyte percentage and the peaks in the red cell count.

[1] Extensive use of cardiac puncture in this laboratory has shown that, if properly done, daily samples of blood (about 0.5 cc.) may be withdrawn over a period of several weeks, without affecting the reticulocyte count or causing marked intra-pericardial hemorrhage.

In stained films as well as in vitally stained preparations, normoblasts in different stages of division are seen after two weeks of cobalt injections. A marked anisocytosis is apparent in plasma, citrate, and saline suspensions of red cells, as well as in the dried films. Polychromasia is very marked after one week. The reticulocytes are not only numerous but a large percentage of these are completely filled with the reticular network characteristic of early forms. The red cell picture resembles that of an anemic animal (Ponder, 1933) except for the red cell numbers, as well as that of an animal in which hemopoiesis is induced by continued low pressure or reduced oxygen tension (Dubin, 1934).

FIG. 1 A

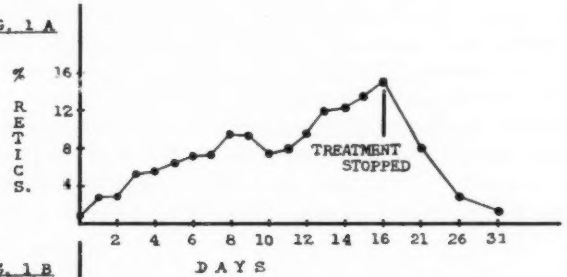
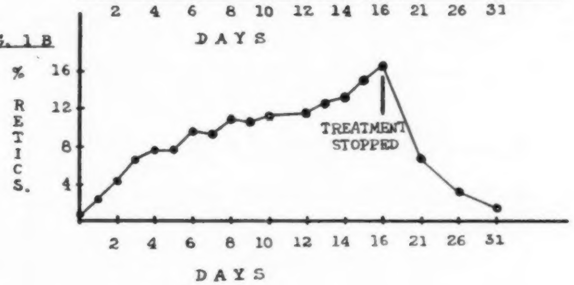


FIG. 1 B

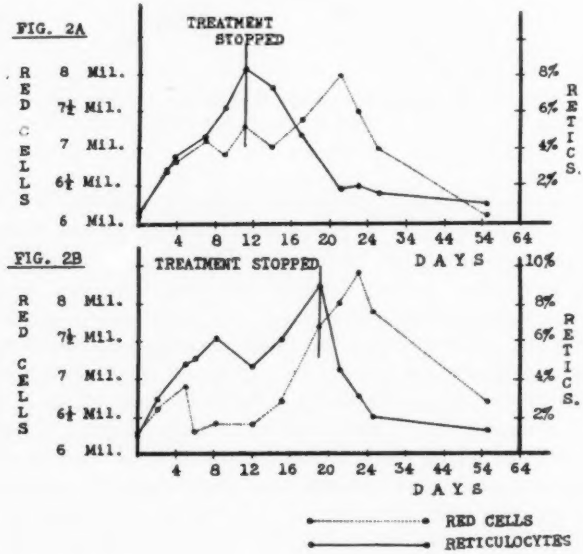


The total number of white cells shows great variation, the mean being within the normal range. A transient lymphocytosis and usually an increase in the total number of white cells follows the first injection and lasts from ten to twenty hours. Similar increases, becoming progressively smaller, follow subsequent injections. A left handed polynuclear count is most marked after the first two injections, the animals thereafter becoming progressively less responsive. Staining of the polymorphs, normally difficult, becomes increasingly difficult because of the development of a "true" eosinophilia. This is evident in the production of large, deeply staining granules which tend to obscure the nuclear configuration. The percentage of basophils is increased and early forms of lymphocytes are seen after the first week of injection. The white cell picture is much the same as that found in rabbits recovering from experimental anemia.

The injections of cobalt were stopped at about sixteen days when the animals began to show a loss of appetite. Animals in which injections were continued developed a diarrhea and died at about twenty-one days. In those cases in which injections were stopped, the animals regained their appetite and did not develop any externally apparent symptoms for a period of two months following treatment. The normal figure for reticulocytes was regained in about two weeks, thereafter the animals showing slight "bursts" in reticulocyte formation, which became less frequent until they could not be detected.

C. *Cobalt and manganese.* The same method was used for the investigation of the effect of manganese upon the hemopoietic symptoms produced by cobalt. Daily subcutaneous injections of 7 mgm. of cobalt as cobalt chloride and 3 mgm. of manganese as the chloride, were given six rabbits. To check the effect of the manganese, two animals were given subcutaneous injections of 5 mgm. of manganese.

The effect of the cobalt-manganese injections upon the reticulocytes is qualitatively the same though quantitatively less than the treatment with 10 mgm. of cobalt. The preliminary drop in red cells usually does not occur, and the erythrocytes increase with the increase of reticulocyte percentage. The red cell increase is continued for a short time after the injections have been stopped and after the reticulocytes have begun to fall. This is shown in the case of two typical animals in figures 2A and 2B. The



animals treated with manganese alone showed no appreciable rise in reticulocytes.

In the case of injections of cobalt-manganese, the animals showed no symptoms of toxicity for at least a month. Thereafter they began to drop in weight very slowly and to become lethargic.

In all other respects, the cobalt-manganese treated animals resembled the cobalt treated animals.

SUMMARY

Cobalt as the chloride or nitrate has a true hemopoietic effect in the rabbit, when injected subcutaneously. This is shown by a high reticulocyte count, the appearance of dividing normoblasts in the circulating blood, anisocytosis, polychromasia, the development of a "true" eosinophilia, a transient lymphocytosis, a slight basophilia, and a slight lymphopoiesis. The addition of manganese to the cobalt makes the erythropoietic effect of the cobalt more marked by producing an erythremia in addition. The combination of cobalt and manganese seems to be less toxic than equivalent stimulation with cobalt alone.

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WATER ABSORPTION AND ELIMINATION OF FROGS DURING ETHER, NITROUS OXIDE, CHLOROFORM, AND ETHYLENE ANESTHESIA

H. W. NEILD AND A. F. SERRITELLA

From the Department of Physiology of the University of Illinois, Urbana, Illinois

Received for publication March 15, 1934

It has been known for some time that the rate at which water is absorbed by an organism is disturbed to a marked extent during anesthesia. Lillie (1918) found that anesthetized sea-urchin eggs lost their permeability during anesthesia, but regained it when the effect of the anesthetic wore off. He concluded that, "Anesthetics (chloral hydrate, alcohols, urethane, ether) also prevent the increase of permeability, in concentrations which are similar to, but in some cases higher than the concentrations arresting cleavage in the same eggs. Eggs which have undergone the normal increase of permeability following fertilization also show a reverse decrease of permeability to water in solutions of certain anesthetics." Heilbrunn (1925) showed that sea-urchin eggs expanded as readily, or even more readily in the presence of ether than in its absence. Adolph (1930) noticed that when frogs were placed in urethane and chloretone solutions they increased in weight two per cent the first hour, and five and five-tenths the second hour, after which the rate of gain was decreased.

METHOD. Ten frogs were placed in a large covered glass dish which contained a sufficient amount of tapwater partially to cover the frogs. Twenty-four hours later the water was changed and the frogs weighed at intervals of thirty minutes until a constant weight was reached. The anesthetic was then passed into the air above the frogs. The rate at which the anesthetic was administered could be varied by changing the amount of air passing through the anesthetic. The amounts of water absorbed were measured by weighing the frogs in groups of five at fifteen minute intervals. Each frog was lifted from the water and partially dried with a damp cloth to remove the excess water and weighed on a triple beam balance to 0.1 gram. The frogs were then returned to the water until the next weighing. The results of each anesthetic used were checked with three groups of frogs. Complete anesthesia was taken to be present when the frogs no longer responded to a tetanic stimulus which had been effective before the frogs were anesthetized. During incomplete anesthesia the frogs responded to the stimulus, but had no volition.

Results. *Ether anesthesia.* The ether air mixture was passed into the covered dish at such a rate that the frogs were completely anesthetized in thirty minutes. During this time and the time following until recovery a marked melanophore effect was noticed. There was little apparent discomfort or excitement. The results of the experiment are shown in figure 1. At the point indicated by letter *a*, complete anesthesia had

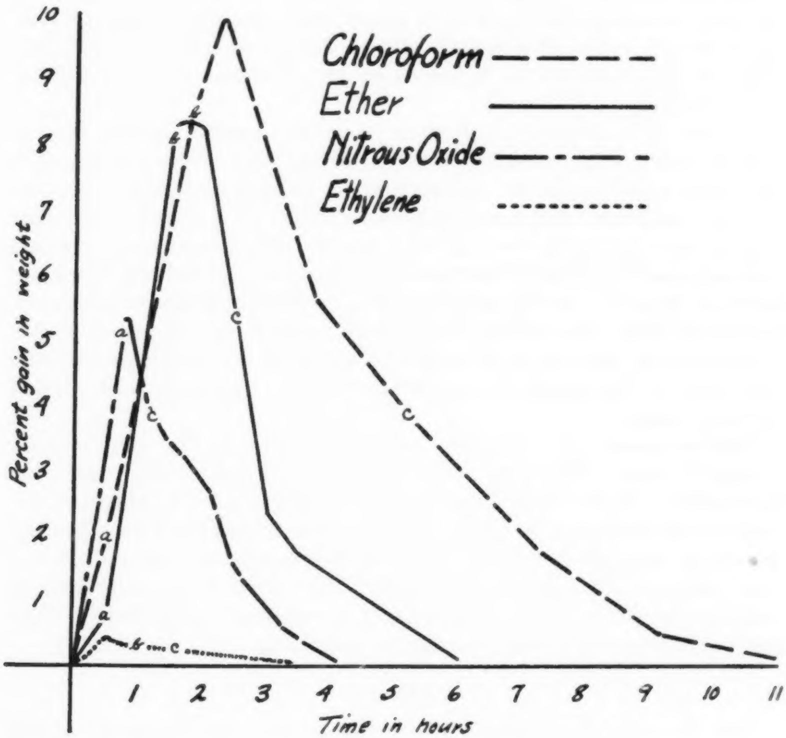

Fig. 1

taken place. At this time the anesthetic was discontinued and the water changed. Letter *b* indicates the time that the frogs were entering the state of incomplete anesthesia. Letter *c* indicates the time when all noticeable effects of the anesthetic had left. Complete anesthesia existed from *a*–*b* (1 hour), and incomplete anesthesia from *b*–*c* (1 hour). If it could be considered that a partial state of anesthesia were present until the frogs reached their constant weight, then the effect of the anesthetic lasted for six hours.

Chloroform anesthesia. The chloroform air mixture was passed into the chamber which contained the frogs in the same manner as ether, and at such a rate that the frogs were completely anesthetized in thirty minutes. The frogs showed a marked melanophore effect and slight discomfort. The frogs showed a moderate amount of excitation. The results of the experiment are shown in figure 1. At the point indicated by letter *a*, complete anesthesia had taken place. Letter *b* indicates the time that the frogs were entering the state of incomplete anesthesia. Letter *c* indicates the time when all noticeable effects of the anesthetic had left. The effect of the anesthetic as indicated by the increased weight lasted for eleven hours.

Nitrous oxide anesthesia. Nitrous oxide was passed directly from a cylinder into the chamber which contained the frogs at such a rate that the frogs were completely anesthetized in forty-five minutes. At this time the anesthetic was discontinued and the water changed. The frogs were completely anesthetized for two minutes. There was marked discomfort and a prolonged period of excitation. The results of the experiment are shown in figure 1. At the point indicated by letter *a*, complete anesthesia had taken place; incomplete anesthesia followed in two minutes. Letter *c* indicates the time when all noticeable effects of the anesthetic had left. The effect of the anesthetic, as indicated by the increased weight, lasted for four hours.

Ethylene anesthesia. Ethylene was administered in the same manner as nitrous oxide. The frogs showed a slight melanophore effect and some discomfort. There was a marked stage of excitation. The results of the experiment are shown in figure 1. Letter *b* indicates the time when the anesthetic was discontinued Letter *c* indicates the time when all noticeable effects of the anesthetic had left. The effect of the anesthetic as indicated by the increased weight, lasted for three and a half hours. The frogs never reached a stage of complete anesthesia.

SUMMARY

The amount of water that was absorbed by the frogs during anesthesia seems to be directly proportional to the hypnotic power of the anesthetic.

The rate at which water was eliminated after the anesthetic was discontinued seems to be directly proportional to the toxicity of the anesthetic.

In all cases water absorption was increased during any degree of anesthesia.

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HEMOGLOBIN AND ERYTHROCYTE DIFFERENCES ACCORDING TO SEX AND SEASON IN DOVES AND PIGEONS

OSCAR RIDDLE AND PELA FAY BRAUCHER

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication March 29, 1934

Though numerous studies on the oxygen carriers of the blood have been made during half a century it is a remarkable fact that as yet we definitely know the nature and extent of sex differences in hemoglobin and erythrocytes in only two species—man and fowl—and that the nature and extent of seasonal variations in these oxygen carriers are definitely known in no species whatsoever. The present study attempts to supply these two kinds of information for two species of birds upon which other concurrent related studies make it specially desirable to know the influence of the sex and seasonal factors. The related studies, upon which variations in the oxygen carriers may be expected to have a bearing, are concerned with seasonal changes in the thyroid glands, basal metabolism, and with the rôle of light (ultra violet particularly) in seasonal changes in the respiratory metabolism. It is again remarkable that despite many excellent studies on these subjects we do not definitely know the effect of season on the basal heat production of man or of animals, and that the effect of light upon either the metabolism or the oxygen carriers still remains in some doubt.

Individual hemoglobin (oxygen capacity) measurements involve a slight error and single erythrocyte counts involve a much larger error which must be overcome by relatively large numbers of closely checked determinations. The fact that diet influences the level of the oxygen carriers and that in most animals and man the diet is not uniform throughout the year makes the measurement of changes due solely to the astronomical progression of the seasons a most difficult task in the case of most organisms. The doves and pigeons used in this study, however, feed upon the same diet of dry mixed grain at all seasons of the year; at least they are provided with an identical grain-mixture at all seasons, and we have failed to note the selection or rejection of any particular grain at any season. Race or strain within a species may provide a further and fairly disastrous source of variability of the oxygen carriers if measurements are not made on genetically known material. All of the races used in the present study were long inbred (hybrids were less inbred) and most of the strains used were "endocrine races" established under selection in this colony. During

maturity these birds were confined in pairs to two-cubic-meter cages; glass walls excluded ultra violet light from November to May, and some heat was supplied during most of this same period. Only healthy adult birds—6 to 30 months old—are primarily concerned in this study and full records of reproduction, together with autopsies done immediately on all birds used

TABLE 1

Mean values (for four seasons) of hemoglobin (grams per 100 cc. blood) and erythrocytes (thousands) obtained for males and females of 7 races of pigeons and for 11 races of doves

RACE	MALES				FEMALES			
	Birds	Hb	Birds	Cells	Birds	Hb	Birds	Cells
				Pigeons				
T	18	15.56	43	3218	20	14.40	43	3082
T-67	10	15.88	17	3140	12	14.44	22	3105
M-T	24	15.10	30	3196	33	14.66	43	3112
T-33	28	16.62	38	3382	23	14.90	36	3176
67+	21	16.80	35	3417	19	15.46	29	3250
R-33	28	15.97	45	3156	21	15.11	37	3012
Misc	47	15.77	73	3088	40	14.05	60	2937
Means	(176)	15.97	(281)	3228	(168)	14.72	(270)	3096
				Doves				
N2+	27	15.66	47	3083	18	14.49	34	2937
62+	26	14.25	50	3004	18	13.76	38	3074
51	14	14.85	25	2909	11	14.48	17	2812
63+	30	14.43	54	3025	26	14.35	54	3024
Na+	21	14.49	40	3094	20	13.73	49	3026
B	13	13.64	16	3052	12	13.78	18	3072
72+	22	14.81	47	3119	19	13.84	42	3105
75	9	14.47	18	3170	13	13.58	24	2924
72-OT+	36	14.74	60	3067	31	14.46	66	3031
63-62+	33	14.03	55	2930	19	13.62	41	2967
Misc	28	14.84	63	3040	19	13.68	46	2902
Means	(259)	14.56	(475)	3045	(206)	13.97	(429)	2989

in hemoglobin measurements, assured the separation of all birds with frank disease from the birds considered here.

With these advantages in this material we believe that the nature and value of the sex factor has been rather definitely determined in both doves and pigeons, and that conclusive data concerning the extent of seasonal changes of hemoglobin and erythrocytes have here been obtained. Some light is thrown on still other related topics. Only the results of several

concurrent studies can supply a basis for conclusions concerning a correlation of seasonal changes in oxygen carriers with changes in thyroids, respiratory metabolism and seasonal irradiation.

METHODS. Hemoglobin was calculated from oxygen capacity as this was determined by the method and apparatus of Van Slyke and Neill (1). Two to 5 cc. of carotid blood from the decapitated bird (with esophagus held aside uncut) were collected in a small beaker containing 2 to 4 mgm. of heparin; 1 cc. of the aerated sample was used in measurement. Non-fasted birds were killed between 11:00 a.m. and 4:00 p.m. during nearly every week of the year for a two-year period.

Erythrocyte counts were made in a Levy-Hausser counting chamber having a glass slide with two sets of Neubauer rulings, and using red cell pipettes of the Thoma type standardized by the U. S. Bureau of Standards. For staining the erythrocytes we used Forkner's (2) modification of Blain's (3) technique. Though these red cells may show marked variation in size we consider them readily identifiable. With this staining method we, like Kennedy and Climenko (4), fail to find the thrombocytes which Fritsch (5) and others report in very small numbers from pigeon's blood. The drop or two of peripheral blood was obtained by puncture of the radial vein with a surgeon's needle after brushing aside a feather or two from the proximal end of the radius of the right wing, moistening and cleaning the bared skin with 25 per cent alcohol, and drying with cotton. This sample for cell counts was usually taken about 30 minutes before the bird was decapitated to obtain blood for the hemoglobin measurement. Duplicate counts, done in accord with Stitt (6), were made on nearly twice as many birds as were used for hemoglobin study.

RESULTS. *The sex factor.* An abstract has previously stated (7, 8) the clear influence of the sex and seasonal factors in these birds. The data (table 1) indicate a mean hemoglobin concentration 8.5 per cent higher in healthy adult male than in female pigeons. Racial means at all seasons show higher male values, and 27 of 28 possible group comparisons (7 races, and 4 seasons studied in both sexes) show higher hemoglobin values in the male. This satisfactorily demonstrates a sex difference in the pigeons studied by us. Similarly the data of table 1 indicate a 4.3 per cent excess of erythrocytes in the blood of males of these same pigeon races. At all of the four seasons higher mean values are found, and 22 of the 28 individual groups show excess male values.

The ring doves show a sex difference of similar kind but of lesser degree. A mean hemoglobin concentration in males 4.2 per cent higher than that of females is found. The general result is repeated in all of the four seasons, and in 34 of the 44 possible comparisons. The data (table 1) indicate that the mean erythrocyte number of males is 2 per cent greater than that of females. These higher mean values extend to all of the four seasons, and

to 29 of the 44 possible comparisons among groups. These results establish a very high probability that the hemoglobin and red cells were present in excess in the male ring doves studied by us.

The seasonal factor. Values obtained during December, January and February are "winter" values. If season influences hemoglobin concentration similarly in the two sexes—and the tabulated data indicate that this is true for both of the species studied—our best measure of the effect of season is obtained by combining the mean male and female values for each season (and division by 2). This gives for common pigeons (table 2) hemoglobin values (winter, spring, summer, autumn) of 15.62, 15.29, 15.13 and 15.34; and for ring doves 14.55, 14.33, 13.83, and 14.34 grams per 100 cc. of blood. • Though the observed seasonal differences are not large they are notably consistent in the two species studied. Lowest values are found in summer and highest in winter in both species. In pigeons the winter

TABLE 2

Hemoglobin (grams per 100 cc.) in blood of male and female healthy adult common pigeons (means for 7 races) and ring doves (means for 11 races) at the four seasons

SPECIES	SEX	WINTER		SPRING		SUMMER		AUTUMN	
		Tests	Hb	Tests	Hb	Tests	Hb	Tests	Hb
Pigeon	♂♂	34	15.92	44	16.11	64	15.78	34	16.08
	♀♀	27	15.32	56	14.47	55	14.48	30	14.59
Dove	♂♂	57	14.86	47	14.66	118	14.17	37	14.55
	♀♀	39	14.25	39	13.99	92	13.52	36	14.13
Mean for seasons		(157)	15.09	(186)	14.81	(329)	14.49	(137)	14.84

value is 3.2 per cent, the autumn value 1.4 per cent, in excess of the summer value. In ring doves—a species not adjusted to cold climates—these percentages are 6.0 and 3.7 in excess of the summer value. Hemoglobin concentration in spring exceeds that of summer by 0.9 per cent in pigeons and by 3.5 in doves. The differences between summer and *autumn* are perhaps of special interest because during these two seasons these birds were exposed to ultra violet light; during winter and two-thirds of spring ultra violet was excluded by walls of ordinary glass.

It is here notable that the species (pigeon) which is native to cold climates has a mean annual hemoglobin concentration 7.8 per cent higher than the species (dove) which is native to a milder climate; also that the hemoglobin of the species which is "naturally adapted" to cold undergoes *less pronounced change* of concentration during cold weather than does that of the dove which naturally avoids low temperature.

If season influences erythrocyte number similarly in the two sexes—and

the tabulated data indicate that this is true for both of the species studied—our best measure of the effects of season is obtained (as in the case of hemoglobin) by combining the mean male and female values for each season. For pigeons (table 3) we thus obtain erythrocyte counts of 3,166,500 (winter), 3,200,500 (spring), 2,991,500 (summer) and 3,289,500 (autumn); for ring doves these counts are 3,052,000, 3,013,000, 2,867,500 and 3,135,500. Seasonal differences of quite similar nature are found in the two species and indeed in both sexes of each species. In all of these larger subdivisions of the data the autumn counts are highest and the summer counts lowest. During autumn these birds were exposed to air of greatest cooling power (as well as to ultra violet light). In pigeons the autumn cell counts are 10.0 per cent, and those of winter 5.8 per cent in excess of the summer counts; in doves—the cold-avoiding species—these seasons show 9.4 per

TABLE 3

Erythrocyte number (thousands) in blood of male and female healthy adult pigeons (means for 7 races) and ring doves (means for 11 races) at the four seasons

SPECIES	SEX	WINTER		SPRING		SUMMER		AUTUMN	
		Birds	Cells	Birds	Cells	Birds	Cells	Birds	Cells
Pigeon	♂♂	63	3178	83	3274	78	3092	57	3368
	♀♀	51	3155	95	3127	71	2891	53	3211
Dove	♂♂	111	3059	85	3071	168	2909	111	3145
	♀♀	94	3045	76	2955	151	2828	108	3126
Mean for seasons		(319)	3109	(339)	3107	(468)	2930	(329)	3213

cent and 6.4 more cells than during summer. The mean annual erythrocyte count of the cold-adapted species (pigeon) is 4.7 per cent in excess of that of its relative (with which it is partly fertile in crosses) from milder climates; but the percentage increase of red cells, as a response to the autumn and winter conditions to which they were subjected, was approximately the same in the two species.

The race factor. That the blood of one of the two species studied (*Columba domestica*) has a consistently higher level of hemoglobin and erythrocytes than the blood of the other species (*Streptopelia risoria*) has already been noted. Though our measurements of particular *races* involve few determinations with high probable errors we should cite evidence that certain races or strains within each of these species are characterized by especially high or low levels of hemoglobin and red cells. We do here find evidence that racial genetic difference extends to the oxygen carriers of the blood.

The most marked racial variant encountered among the pigeons was

otherwise characterized by hereditary ataxia. It is thus excluded from the "normal" races considered in this paper. The study of this race has been continued and will be reported elsewhere (Riddle and Bates). In table 1 it will be observed that Race 67 showed, in both sexes, the highest concentration of hemoglobin. Our fully tabulated data—giving data for each race (both sexes) at all of the four seasons—would require more space than journals would grant, but we may note that such data show that at all of the four seasons, and in both sexes, the values found for this race exceeded the values found for any other race. Likewise the complete data (and the arithmetic means given in table 1) supply almost equally uniform evidence that Race 67 also has the highest cell count of the several races examined. Probably the lowest hemoglobin values characterize the "miscellaneous" group which includes such very large races as the Runts and White Kings and various hybrids of these large races; this same group also had the lowest erythrocyte count of the races studied.

Among the ring doves Race N2—both males and females—has blood of highest mean annual hemoglobin content, though its blood count scarcely differs from the means found for the several races. Race N2 was selectively established in our colony (9), with relatively and absolutely longer intestines than any other race within the colony. Lowest hemoglobin values are perhaps associated with Race B—which has the largest (hypofunctional) thyroids of the several races of our colony. Race 51 is rather plainly indicated as having the lowest erythrocyte count; its hemoglobin value is apparently nearly or quite normal. The normal individuals of this race carry a recessive genetic factor for myoclonus and are further characterized by an aberrant body temperature.

These results on the race factor, even in the unfinished state of our studies on these races, provide a few significant associations of endocrine and constitutional factors with high or low level of oxygen carriers in healthy individuals. They also convincingly show that genetically known animals should be used in such studies as involve the measurement of sex or seasonal factors on the oxygen carriers of the blood. Otherwise the use at various seasons of genetically dissimilar animals, or of the same mixed group in unequal proportions at different seasons, could unwittingly obscure true seasonal variations and even suggest spurious ones.

The age factor. From the above tabulations data for age are omitted, though the age of every animal used was exactly known and recorded. The following data, obtained especially to test the influence of the age factor, seem to show that among birds aged 6 to 30 months age is not a significant factor, since values then obtained are but slightly less than those obtained in immature birds aged 2.5 to 4.0 months. For 12 immature male pigeons we find 16.23 grams hemoglobin and 3,343,600 cells; for 14 such females these values were 15.34 and 3,281,500. For 10 immature

male ring doves 14.01 grams hemoglobin and 3,027,000 cells were obtained, the values for 14 females being 13.66 and 3,082,000. When the means of these values are properly combined, with race, sex and season considered, it is found that the blood of these young birds had 14.81 grams hemoglobin and 3,199,050 cells as compared with 14.22 grams hemoglobin and 3,072,200 cells found in a similar number of the fully mature birds. Since no old birds (they live 6 to 12 years) and also none younger than 6 months are included in tables 1 to 3 it appears permissible to consider those birds a single age-group. It is true that the basal metabolism of birds aged 2 to 4 months has been found by various workers to be definitely higher than at any later stage; and it is perhaps significant that birds of this age are here found to have slightly higher hemoglobin and cell counts than are found in the mature birds.

Oxygen carriers in sexually abnormal females. Among the 465 healthy normal adult ring doves and the 344 healthy normal adult pigeons described here we found 16 doves and 14 pigeons aged 12 to 23 months which had never produced an egg, but which at autopsy proved to have ovaries. All of these birds were apparently healthy and if normally feminine should have produced eggs when aged 4 to 10 months. The distinctively feminine function of egg-production was either temporarily or permanently suppressed in these 30 birds. Since hemoglobin and erythrocytes were measured also in brothers and sisters (or at least in birds of the same inbred race) of these "unfeminine" females we here have opportunity to learn whether and in what respects the oxygen carriers were abnormal in these delinquent birds. Data for comparison are given in table 4.

TABLE 4

Hemoglobin (grams per cc.) and erythrocytes (thousands) of apparently healthy female pigeons which became sexually mature late (or never) in life compared with values obtained from normally maturing females and males of the same race and season

SPECIES	"RETARDED" FEMALES			ADULT FEMALES OF SAME RACE AND AT SAME SEASON			ADULT MALES OF SAME RACE AND AT SAME SEASON		
	No.	Hb	Cells	No.	Hb	Cells	No.	Hb.	Cells
Doves	16	15.36	3099	32	13.87	2932	54	14.50	3074
Pigeons	14	15.34	3217	52	14.76	3114	64	15.96	3216

Considered as a group the 16 "retarded" female doves had 10.6 per cent more hemoglobin and 5.7 per cent more red cells than their normally feminine sisters, and these values were even 5.9 and 0.8 per cent higher than corresponding values in their brothers. There was but one bird of this group with low oxygen carrier values; and these values were so extraordinarily low (hemoglobin 10.03; cells, 2,096,000) as to provide our only evi-

dence of disease (see below) in this bird. The group of 14 "retarded" pigeons had 3.9 per cent more hemoglobin and 3.3 per cent more erythrocytes than their normal sisters, and these values equalled or closely approached those of their brothers. Within this group also only a single bird showed low values (hemoglobin 13.43; cells, 2,588,000). It is notable that the inclusion of data from such "unfeminine" females with data for typical normal females (as is done in our tables 1–3) serves somewhat to minimize the real differences between males and true females. There is reason to believe that similar departures from normal sexuality exist among the females of most or all other animal types.

Surely in these two groups of cases there was suppression of egg-production associated with oxygen carriers at or above the male level. To whatever extent the oxygen carriers here reflect the relative oxygen demands of the tissues the somatic tissues of these two groups of "retarded" females are indicated as having oxidation rates more like those of their brothers than of their sisters. These measurements, like many others made by the senior author on "masculinized" females during the past 20 years, again accentuate the quantitative nature of sexuality. We consider these "females" as true intersexes, and though it is not known whether this intersexuality was of genetic or environmental origin that point is unimportant here.

Usual effect of disease. Autopsy revealed disease in 61 of the 903 supposedly healthy doves and pigeons taken for hemoglobin measurement. Analysis of the data from the diseased birds shows that all distinctions of age and race—even of species difference—here disappear. Probably seasonal differences also disappeared, though summer and autumn show lowest values for both hemoglobin and cells. Mean values for all males (species separated and assigned seasonal value) are 12.65 grams hemoglobin and 2,708,200 cells; for females, 12.42 and 2,863,200. All of these mean values are lower than those normal for any race included in this study. Though not every individual showed reduction of the oxygen carriers this was the obvious rule, and there can be no doubt that failure to identify and eliminate diseased doves and pigeons by autopsy would lead to average values lower than those truly characteristic of healthy individuals.

Effects of special conditions, particularly of thymobursectomy and close-caging. Table 5 supplies data on these points. The removal of both the thymus and the bursa Fabricius (thymobursectomy) at the early age of two months, with measurements made 7 to 16 months later, led to an apparent increase in the cell count in all of 7 tests (average 16 per cent). The hemoglobin—at least the oxygen-carrying hemoglobin—was probably not increased. The increased cell count, however, is similar to that found by Toryu (10) in a carrier pigeon 7 weeks after splenectomy. In that study, as others had found after splenectomy of certain mammals, 1 to 25 per cent

of the total hemoglobin was of the non-oxygen-carrying type. Our data suggest that thymobursectomy—done in very young birds—after a pro-

TABLE 5

Special conditions in doves and pigeons tested for effects on hemoglobin and cell count

OPERATION		TIME SINCE OPERATION	HEMOGLOBIN		CELL COUNT	
Or condition	At age		Test	Control*	Test	Control*
Pigeons						
	mos.	*mos.*	*grams per 100 cc.*		*thousands*	
Thymobursectomy	1.7	11.4			4328	3237
Thymobursectomy	1.8	16.5	14.25	13.96	3700	3496
Thymobursectomy	1.9	8.4			4176	3363
Thymobursectomy	2.0	16.2	15.63	15.30	3800	3140
Thymobursectomy	2.0	10.0			4104	3363
Thymobursectomy	2.1	10.6			3760	3546
Thymobursectomy	2.2	6.6			3296	3237
Thymectomy**	7.9	12.9	15.26	15.43	3050	2784
Thymectomy	12.5	12.9	15.72	15.39	2702	3058
Thymectomy	13.7	12.9	13.82	15.91	2877	2869
In very small cages		1			3336	3335
In very small cages		1			3632	3335
In very small cages		1			3320	3470
In very small cages		1			2560	3295
In very small cages		1			3664	3295
In very small cages		1			3640	3542
Could never fly	16.4	0			3988	2985
Ring doves						
Myoclonus (?) + ataxia		49.6	20.60	16.03	4112	3291
Cross-beak		15.4	14.90	14.11	3896	3119
In very small cages		1	14.98	14.85	3184	3127
In very small cages		1	15.34	14.11	3540	3119
In very small cages		1	15.08	14.70	3396	2899
In very small cages		1	14.51	13.47	3532	2992
In very small cages		1			3784	3154
In very small cages		1			3576	3135
In very small cages		1			3288	3058
In very small cages		1			3608	3085
In very small cages		1			4032	3107

* Each "control" value was obtained from a group of adult birds of the same race and sex and at the same season.

** An unsuccessful attempt by O. Riddle and Dr. R. W. Bates to duplicate the preceding results by thymectomy alone in adults.

longed period leads to an increased red cell count as splenectomy has been reported to do in the pigeon (Toryu) and as others had earlier found in

mammals. More recently Riddle and Bates sought results from pigeons on which simple thymectomy was performed in the adult stage (when the bursa normally should have atrophied). Only 3 birds were alive when measurements were made 13 months later, but clearly no effects were obtained with this modification of the earlier procedure. This subject deserves further study.

Various laboratories provide unequal space for pigeons with which they chance to work. We wished to know whether confinement of pairs of birds during one month to one cubic foot of space—instead of the usual 70 cubic feet—would affect the quantity of oxygen carriers. This degree of confinement lowers the blood sugar (11) and decreases the basal metabolism (12). The evidence here obtained indicates no consistent change in cell count due to close-caging in pigeons, but a regular (all of 9 tests) increase of the count in doves (average, 15 per cent). Four tests for effects on hemoglobin in doves all suggest a small increase (5 per cent). Curiously in line with this unexpected effect of close-caging in doves, however, are cases of a pigeon which throughout life had been unable to fly, of a dove with complicated myoclonus, and of a dove whose cross-beak made eating so difficult as to make under-nutrition presumable. In all three cases extraordinarily high cell counts were obtained. In doves subjected to close-caging it is found that the erythrocytes may increase coincident with a decrease in blood sugar and basal metabolism.

Literature and comment. Hemoglobin measurements and cell counts on pigeons seem to have been done only by Barlow and Whitehead (13) and by Toryu (10). The American workers were only incidentally concerned with the oxygen carrier values obtained on 68 normal pigeons; the sexes were not separately reported and averages were given for neither cell count (range, 3,780,000 to 4,530,000) nor hemoglobin. The Japanese study was concerned with effects of splenectomy in a very few pigeons. Most earlier erythrocyte counts are almost negligible. De Eds (14) reported 25 counts made on heart blood (3,300,000) and 26 on blood from the leg vein (3,350,000), and noted a high variability which is confirmed by all later work including our own; the sex was not stated. We consider sex, season, race, disease, diet, excitability, exercise, condition of caging (space and light) and different staining techniques as making for high variability, and some of these factors as responsible also for the very high average values (3,800,000 cells) found in the fragmentary earlier work. Our mated birds had little fear of us, and could be caught with little flight and fright. A probable slight dilution of the blood in autumn and winter, incident to bringing the animals into (higher) laboratory temperatures, could not be avoided. Stage of the reproductive cycle seems to make no difference in the cell count of these two species (200 tests), but practically all of the data reported here were obtained at "resting" and "ovulation" stages of the cycle.

Blacker (15) and Dukes and Schwarte (16) have shown that in the fowl both hemoglobin and erythrocytes exist in greater quantity in male blood; several recent studies have confirmed this sex difference in the cell count. Otherwise so far as we are aware, it is only in man that the available data satisfactorily fix the sex status of relative hemoglobin concentration and erythrocyte number. For many species the data at hand suggest higher values in males (Scarborough, 17); convincing data for several species are highly desirable.

Effects of race or breed on the oxygen carriers has been several times reported in animals. After the present study was started Bobroff and Brener (18) published data for man indicating the existence of "constitutional" types in which the average (2217 boys, 175 girls) hemoglobin values differ. Our study, based in part on races in which certain hereditary endocrine differences are known, confirms and extends those results. Certain genetic differences which are here found to accompany different levels of the oxygen carriers are concerned with size and functional level of certain organs already reported (19, 20), and with basal metabolic rates still under study.

A seasonal variation of erythrocyte number in cattle (21), with higher winter and lower summer values, and a similar variation in the hemoglobin of the guinea pig (22) have been reported. A group of New York children aged 21 to 44 months, measured from November to May, showed lowest hemoglobin values in February (23). Seasonal effects, and specific effects of total light and of ultra violet light, have been frequently reported for man; the results, however, are contradictory or indecisive—and this in the species most capable of making its own intimate climate so far as temperature is concerned. While light is rightly regarded as probably the most important seasonal or climatic factor for man, temperature may prove to be of equal or greater importance to many higher animals. In a well-planned study Nylin (24) concurrently studied the effects of season and light on the growth, basal metabolism and hemoglobin of children. The oxygen capacity showed periodicity, with a winter minimum and a summer maximum, while high and low points in the standard metabolism preceded those for oxygen capacity by several months.

Of the concurrent studies made on the material presented here only one item—seasonal changes in the basal metabolism of tippler pigeons—has yet been published (25). It was there found (for race T of table 1) that the metabolism (at 20°C.) is highest in autumn—when erythrocytes are at a maximum, and just previous to the maximum of hemoglobin; and lowest in spring or winter (period of no exposure to ultra violet light) when neither their hemoglobin nor their erythrocytes are apparently most diminished. Obviously the basal metabolism of other races, and results of still other current studies on all our dove and pigeon races, should reveal the extent

to which certain other functional levels vary in consonance with the marked seasonal changes in oxygen carriers which are demonstrated in the present study.

SUMMARY

By the oxygen capacity method a total of 931 hemoglobin measurements was made throughout the year on suitably inbred races of the pigeon and the ring dove—two species on which 1583 erythrocyte counts were simultaneously made.

The blood of healthy adult males of both species was found to have a higher concentration of hemoglobin at all seasons than that of females. Mean values were 15.97 grams in males and 14.72 grams in females (pigeons); and 14.56 grams in males and 13.97 grams in females (doves).

The blood of healthy adult males of both species was similarly found to have a higher erythrocyte count at all seasons than that of females. Mean values were 3,228,000 in males and 3,096,000 in females (pigeons); and 3,045,000 in males and 2,989,000 in females (doves).

These birds were deprived of ultra violet light from November to May and were then provided with some heat. Hemoglobin values were highest in winter—when the birds were exposed neither to air of greatest cooling power (autumn) nor to ultra violet light. Lowest values were found in summer—when in air of least cooling power and well exposed to ultra violet rays. In pigeons the winter value exceeds that of summer by 3.2 per cent; in doves, by 5.2 per cent. Autumn values exceed those of summer.

Highest erythrocyte counts were found in autumn, lowest in summer. In pigeons the autumn excess is 10 per cent; in doves, 9.4 per cent. Thus both hemoglobin and cells are reduced—though unequally—to their lowest value at the hot season.

Within each of the two species studied certain races appear to have abnormally high or low levels of oxygen carriers of the blood in association with endocrine differences already known to characterize these races. We find little or no evidence that a race with high or low hemoglobin necessarily has a correspondingly high or low cell count.

Two groups (30 individuals) of females, which we regard as intersexes, attained advanced age with persistently inactive ovaries and were then found to resemble their brothers more than their sisters in hemoglobin concentration and cell count.

Seven tests indicate that the extirpation of the thymus and bursa Fabricius from very young pigeons leads, after several months, to a cell count 16 per cent higher than normal—a result which others have observed following splenectomy in birds and mammals.

Hemoglobin and erythrocytes are only slightly higher in adolescent birds

than in adults; both are usually markedly reduced in diseased birds. The cell count of doves is increased by close-caging for one month although other studies have shown that by this means blood sugar and respiratory metabolism are slightly decreased.

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DIURNAL CHANGES IN THE LIVER DURING PREGNANCY

THOMAS W. GOODWIN AND GEORGE M. HIGGINS

From the Mayo Foundation and the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota

Received for publication February 8, 1934

Forsgren described a cyclic activity in the glycogen-forming and bile-secreting functions of the liver, and he designated these alternating functions as the assimilatory and secretory phases of the cycle. Agren, Wilander, and Jorpes regarded the cyclic changes that occurred in hepatic glycogen as largely independent of food intake; and they demonstrated that glycogen was present during the night and disappeared from the liver during the day. Higgins, Berkson, and Flock studied the changes that occurred in the livers of white rats after a single, restricted feeding-period. They indicated that there were definite cyclic changes, not only in the glycogen content but also in the water, protein, and, to a less extent, fat content of the liver following a single feeding. Furthermore, the curve describing these daily changes was definitely bimodal.

Contrary to the opinions of Agren, Wilander, and Jorpes (1931) and of Holmquist (1931, 1933), Higgins, Berkson, and Flock (1933) concluded that these cyclic changes in the liver, especially those which concern the glycogen and water content, were dependent on feeding. When experiments were rigidly controlled and rats were trained for prolonged periods to eat at definite times during the day it appeared that the increase in glycogen and water in the liver depended wholly on intake of food and water.

This study was undertaken to determine what effect pregnancy might have on these definite cyclic changes in the liver. Do these daily cyclic changes in the livers of normal rats differ from those in animals early in pregnancy, or in any essential way from those occurring near term? It is reasonable to assume that in late pregnancy, when the fetuses have imposed greater demands on the maternal organs, metabolic changes would be reflected in the maternal liver. Accordingly, the livers of a series of rats at about the eighth or ninth day of pregnancy, and a second series at the eighteenth or nineteenth day, were studied with respect to the water and glycogen changes during a twenty-four hour period following a single feeding.

Although there are considerable data to indicate that changes in the deposition of hepatic glycogen during pregnancy do occur, yet daily

fluctuations in water and glycogen never been have followed. Cahne demonstrated a diminution in hepatic glycogen during the later stages of pregnancy, and Loveland, Maures, and Snyder demonstrated that there was a reduction of as much as 75 per cent in the amount of placental glycogen during the latter third of pregnancy.

Bokelmann and Dieckmann (1930) showed that the glycogen content of the livers of rats was low during proestrus and in early estrus and was high during metestrus and in diestrus. They found that the fat content of the liver during estrus was just the reverse of that of the glycogen content.

Method. Extensive use was made of the complete monograph by Evans and Long (1922) on the estrual cycle in the white rat. Mature females of our colony of white rats, which has been derived from the Wistar strain, were isolated, and vaginal smears were made daily. The date of the last appearance of stage 2 of the cycle, the finding of spermatozoa in the vaginal smear and the interrupting of the estrual cycle, were taken as signs of pregnancy.

All animals were trained to eat and to drink from 9 to 11 each morning for about three days before the day of the experiment. Rats eat by choice during the night, but they may be trained to eat at any time during the day. Food, a standard ration for rats, and water were placed in the cage at 9 a.m. and removed at 11 a.m. each day. All animals then were fasted from 11 a.m. each day to 9 a.m. the following morning so that food was available only for two hours each day.

In order to have control data with which to compare the experimental data, fifteen pregnant rats in each series were killed at 9 a.m. after a twenty-two hour fast. Weights of the bodies and of the livers were recorded and the percentages of water and glycogen in the livers were determined in the manner previously reported by Higgins, Berkson, and Flock. From the data thus assembled, formulas were derived by which the weight of the liver could be computed from the weight of the body, and the amounts of hepatic glycogen and of hepatic water could be computed from the weights of the liver at 9 a.m. In this way the weight of the liver and its glycogen and water content were computed for each pregnant animal at 9 a.m. just before feeding time, and thus by contrasting the actual weight of these constituents at the time the animal was killed with that estimated to be present in that animal at 9:00 o'clock, the gain or loss in each constituent at each two hour period was determined.

The data on the cyclic changes which occurred after feeding were assembled in the following manner: three pregnant animals in each series were killed at 11 a.m. on the day of the experiment, at the time food and water were removed from the cages, and three more were killed at every two-hour interval thereafter until 7 a.m. the following morning. The

mean increase in the weight of the liver and in the amounts of glycogen and water in the liver were computed; when these figures were contrasted with the amounts found at 9 a.m., the differences were recorded as changes for that period during the day.

Experimental observations. The water and glycogen content of the liver were computed in terms of percentage of total hepatic weight. On this basis a fluctuation was revealed in the glycogen content, from 0.1 to 0.2 per cent at the fasting level to 4.5 to 5.0 per cent in the twenty-two hours following feeding. The water content varied from 68 to 77 per cent of the total weight of the liver. In view of the cyclic variation in the weight of the liver during the twenty-two hour period, it was apparent that the expression of the changes in the glycogen and water in per cent did not reveal the changes that actually occurred. The amount of water present at 3 p.m., for example, was vastly greater than the amount of water present at 7 p.m. when the weight of the liver was relatively low, although the actual percentage of water present was essentially the same in relation to the respective weights of the liver at those times. Therefore, the changes observed have been expressed in grams of glycogen, or grams of water, lost or gained at a given period.

On this basis, it appeared that change in the water content of the liver was greater than in the glycogen content and accounted for the greater part of the change in total weight of the liver, roughly 70 to 75 per cent. Glycogen was present in significant amounts at certain hours, however, and produced changes as high as 20 per cent. These changes in grams of liver weight and in grams of glycogen and water content, in each period during the twenty-two hours for each of the two series of animals, are condensed in the accompanying graphs. The curves of the changes which occurred in the livers of maternal animals early in pregnancy (fig. 1) were bimodal and corresponded rather definitely with those described by Higgins, Berkson and Flock. The data assembled from animals near term, however, did not reveal as marked cyclic changes (fig. 2), and yet when the weights of the fetuses and fetal membranes were deducted from the body weights of the pregnant rats, then the curves of the changes were more characteristic (fig. 3).

Comment. In these studies on pregnant animals there were shifts in the significant points on the curves so that the initial mode occurred at 3 p.m. rather than at 5 or at 7 p.m. as observed for the normal. The second mode occurred at 11 p.m. and the low point on the curve at 7 p.m. Accordingly, the curves of changes in livers of pregnant animals agreed very well with those of normal animals, although these changes occurred somewhat earlier in the pregnant group.

When the actual amounts of glycogen and water in livers of eighth to tenth day pregnant animals were compared with those recorded for normal

animals, some interesting contrasts were noted. The mean increases in weights of livers and of glycogen in livers at the initial mode were considerably greater in normal animals, although the increases in weights of water at that time were greater in pregnant animals. At the second mode, however, the total increases in weights of livers were much greater in the pregnant animals, and although the weights of glycogen in livers were less than half that recovered from normal animals, the increases in weights of water were 0.3 gram greater. In other words, the increase in weight of water in the liver recorded at the significant points in the curve was always greater in the pregnant animal, although the increase in weight of glycogen at comparable times was always less than that found in the liver of the normal animal.

The data assembled from the animals late in pregnancy were compiled in two ways. By means of two formulas the changes in weights of livers and of water and glycogen in livers were computed for all animals: 1, when weights of fetuses and fetal membranes were included in the body weight determinations, and 2, when the weights of fetuses and fetal membranes were deducted from the body weights. The curves of the changes (figs. 2 and 3) are obviously unlike, for the varying sizes of litters and of individual fetuses were of sufficient magnitude to introduce considerable variation. When the changes in weights of livers of animals whose body weights included fetal weights were plotted, the resulting graphs were practically straight lines, although there was some slight tendency toward bimodality (fig. 2). The curves of the changes recorded when the uterine contents were disregarded in the computation (fig. 3) appear to possess more than two modes, and yet, statistically, they are definitely bimodal and correspond essentially with those describing the changes at the eighth day of pregnancy. The initial mode was reached at 3 p.m. and the low point on the curve occurred at 5 p.m. An immediate increase in the weight of the liver was recorded so that the second mode on the curve occurred at 7 p.m. and continued until 1 a.m. with fluctuations occurring at 9 and 11 p.m. These points at 9 and 11 p.m., when considered statistically with reference to the changes at 7 p.m. and 1 a.m., are not significant, and the second mode may well be considered a plateau extending from 7 to 1 a.m. The increase which occurred at 5 a.m. is likewise not a significant change.

In late pregnancy, the increase in weight of the liver at the initial mode,

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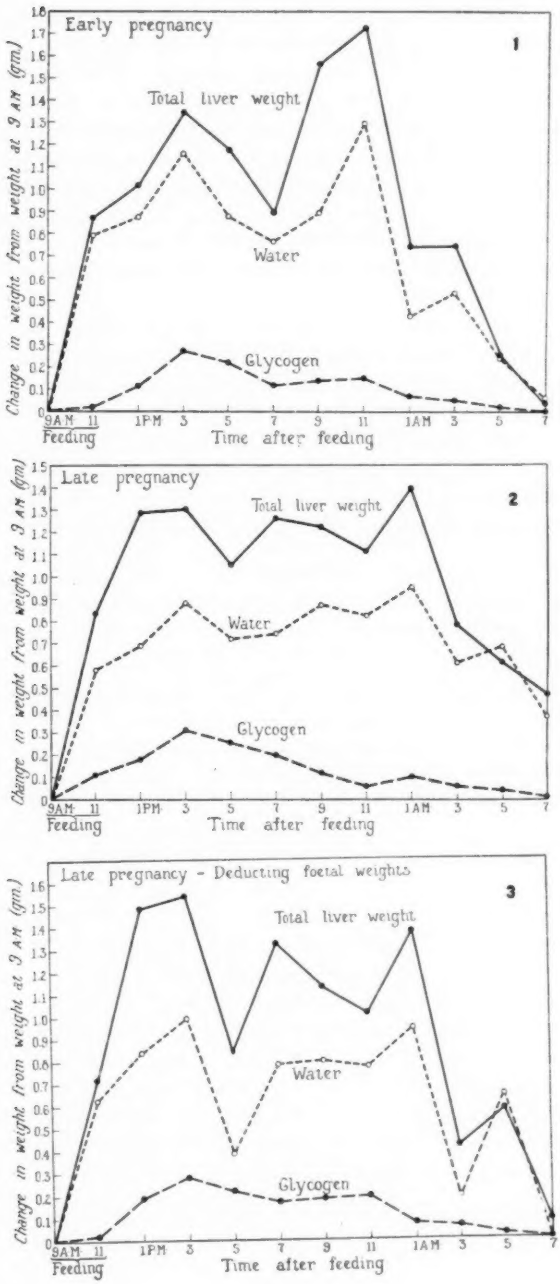
Fig. 1. Curve of changes in weights of livers and of water and glycogen in livers, following a single two hour feeding period, of animals early in pregnancy.

Fig. 2. Curve of changes in weights of livers and of water and glycogen in livers, following a single feeding period of animals near the end of pregnancy.

Fig. 3. Curve of changes in weights of livers, and of water and glycogen in livers, following a single feeding period, of animals near term when fetal weights were deducted from total body weights.


Early pregnancy
1
Total liver weight
Water
Glycogen
Change in weight from weight at 9 AM (gm)
9AM 11 1PM 3 5 7 9 11 1AM 3 5 7
Feeding
Time after feeding
Late pregnancy
2
Total liver weight
Water
Glycogen
Late pregnancy - Deducting foetal weights
3
Total liver weight
Water
Glycogen

Figs. 1-3

3 p.m., was considerably greater than that in early pregnancy, and this increase, 1.540 ± 0.333 gram, corresponded very well with the increase, 1.537 ± 0.053 gram, recorded for normal animals. The increase in weight of water was considerably less than that recorded at early pregnancy, although the determinations for glycogen were essentially alike at both times. In both series of pregnant animals the mean increases in glycogen in livers were lower than those recorded for normal animals. Mean increases in water in livers were less in animals in late pregnancy than in those in early pregnancy and in the control group, a fact probably correlated with the rapidly growing fetuses with their rapid utilization of water.

Accordingly, it is clear that the curves of the changes in weights of livers and of water and glycogen in livers of pregnant animals following a single feeding are bimodal in character and, in general, agree with the curves of such changes in normal animals. Contrasts indicate, however, that 1, the peaks occur somewhat earlier in pregnant animals; 2, the amounts of glycogen recovered were always less in pregnant than in normal animals, and 3, the amounts of water recovered from livers at the peaks of curves were always greater in animals in early pregnancy than in those at term.

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ELECTRICAL MEASUREMENTS CONCERNING MUSCULAR CONTRACTION (TONUS) AND THE CULTIVATION OF RELAXATION IN MAN—RELAXATION-TIMES OF INDIVIDUALS[1]

EDMUND JACOBSON

From the Physiological Laboratory of the University of Chicago

Received for publication February 15, 1934

In 1889 Orschansky reported that following a signal his subjects could relax the masseter muscle in 0.11 to 0.30 second, according to certain conditions. The interval decreased with practice, but increased with fatigue. Upon relaxing, the muscle ceased to press on a key which, being released, opened a circuit and thus operated a signal-magnet. A Marey tambour, actuated by air-pressure from a flexible capsule in the mouth, provided a record indicating amplitude. Orschansky found the "reaction times for contraction" and the "reaction times for relaxation" approximately equal on the average. In 1922 Voerckel reported on times required for relaxation in bending or extending the forearm tested with a string galvanometer and with a lever attached to the middle finger. The mechanical measurements appeared to give shorter times,—but she ascribes this to contraction occurring in the antagonists before relaxation is complete, thereby concealing the endpoint. Measured electrically, the time required for relaxation, e.g., after flexing in one subject, averages about 0.21 second, but ranges approximately from 0.09 to 0.41. Unlike Orschansky, she finds that the average times required for relaxation exceed those for contraction; but since the briefest intervals which she observes for contraction and relaxation do not differ greatly, she deems it probable that with sufficient practice this difference would be eliminated.

These earlier investigations indicate that relaxation can at times be affected approximately as quickly as contraction. The authors agree on the need of practice to effect this approximation, since at first the times required for relaxation are likely to be prolonged and variable. A slight confusion results from their failure to distinguish between the interval from signal to *onset* of relaxation as contrasted with the interval from the same signal to the *completion* of relaxation. When this distinction is made, it becomes obvious that in each test the former and not the latter interval should be compared with "reaction-time for contraction," which means the time to *onset* of contraction. Accordingly for clarity on this point, we shall

[1] This investigation is a member of a series aided by a grant from the Josiah H. Macy Junior Foundation, which is here gratefully acknowledged.

define "relaxation-time" as meaning the interval from the signal to the attainment of complete relaxation.

With the instruments available to the authors mentioned, they could not settle the question whether relaxation is complete in the periods which they determined. More nearly accurate determinations were made in 1930 (Jacobson) with an amplifier-string-galvanometer system, but only concerning the slight muscular contractions present in specific localities during mental activities such as imagining lifting a ten-pound weight. These contractions, we found, can be relaxed in trained subjects in periods varying from 0.2 to 1.5 second, averaging 0.4 second for the group tested.

In the present study, the course of voluntary relaxation is investigated in 14 university students and in 22 patients. With some patients, determinations were made before and after training to relax, since this might furnish further indication whether or not such training is effective.

Methods. The instruments and subjects are the same as in a previous investigation and the determination of relaxation time generally was made after a half-hour record of rest had been taken (Jacobson, 1934). As previously, also, the electrode ("positive") connected with the inner turn of the input transformer is inserted about 1 to 2 cm. into the right biceps-brachial muscle group at a level about 6 to 8 cm. above the other electrode ("negative"), which is inserted subcutaneously in the elbow pit. (Erratum: loc. cit., p. 232, line 11. Delete "grid of the first tube." Insert "inner turn of the input transformer.")

The subject is requested to flex the right forearm slightly at the first click of a telegraph key and to relax as quickly and completely as possible at a second like signal. No preparatory warning or pre-signal is given, except telling the subject to be ready just before a set of tests.

Practise tests are made until it appears that instructions are fully understood. The subject was generally watched to see that the wrist did not move more than a few centimeters. In a few instances, a mechanical as well as electrical record was taken. The interval between the two signals varies, mostly from one to two seconds, but is almost always less than three seconds. Longer time intervals are not employed because they seem to enable some untrained subjects to relax more promptly, obscuring individual differences, which was contrary to our purposes. It is necessary to vary the interval in order to prevent the subject from expecting the second signal at a given moment and therefore tending to relax at a fairly constant interval after the first signal, in place of reacting to the second signal as desired. In a few records some subjects evidently failed to wait for the second signal to relax, for they showed instances where relaxation began before the second signal and other instances where relaxation began in very brief intervals after the second signal (e.g., 0.04 sec.). Such records were discarded. The subjects who made them were informed of the

failure to follow directions and were given practice in proper performance before final records were made.

Generally the photographic record is taken continuously for at least 10 seconds after the second signal. Thereafter, if action-potentials are still above 1.0 microvolt, the camera usually is turned on and off at set intervals for about two minutes. In most instances three or more tests were recorded for each subject, but sometimes a single test seemed a fair sample. It should be mentioned that even the trained subjects had no previous experience in relaxing upon signal. The aim is to study in normal and neurotic individuals the course of action-potentials in early tests following the signal to relax rather than to make statistical determinations of relaxation-times as have the above-mentioned investigators.

As a rule the shunt resistance and the string tension are adjusted so that the excursion of the shadow is 3.5 mm. on either side of the zero-line when a potential difference of one microvolt at 60 cycles is impressed upon the input of the amplifier. In consequence, while the arm is bending after the first signal, the string shadow invariably shoots back and forth beyond the limits of the photographic record. The voltage here is undetermined, excepting that it exceeds 7.1 microvolts both positively and negatively. For statistical purposes, as in the previous investigation, we read only on one side of the zero line, and set down excursions beyond the record as 7.1+ microvolts. In averaging, the plus factor is ignored. Where this is done, the averages obviously do not represent absolute values, but may be used for purposes of comparison. Toward the end of the series when the string shadow passed beyond the limits of the photograph for a number of seconds, the operator quickly changed the shunt resistance so as to diminish the string excursions and permit the microvoltages to be determined, whatever their magnitudes.

Results—gross contractions. *Students not trained to relax.* Figures 1 and 2 are illustrations from records of the second set of tests with each subject. One subject (S.D.) shows relaxation not yet attained even two minutes after the signal has been given. (Similar failure to relax is exhibited in his half-hour record which showed microvoltages ≤ 0.3 for only 51 per cent of the period and mean $V_m = 0.71$.) Another subject (T.N.) relaxes completely 0.8 second after the signal, being the only one of the 14 students tested whose average relaxation time falls under 1.0 second. (Likewise his half-hour record of rest is lower in microvoltage than that of any other student.) Nevertheless, as will be noted in figure 2, he fails to maintain relaxation throughout the period prior to the first signal. Failure in this respect occurs in four out of five tests made with T. N. and frequently with each of the students. As a rule with the 14 students tested relaxation is not so delayed as occurs in figure 1 nor so promptly attained as occurs in figure 2.

In figure 4 is shown for both groups of students the course of relaxation as measured in action-potentials. For group 1, the curve A represents the results in the first set of tests, while the curve A′ represents the results in the tests made a week later. That these curves are close throughout their course indicates that repetition does not reduce relaxation time in "normal" subjects so rapidly as to destroy the usefulness of the test, particularly if the test is not repeated more than three or four times on each occasion (not including the preliminary trials necessary to make sure that the instructions are understood).

In the untrained subjects tested, relaxation commonly occurs more or less abruptly and incompletely at first and generally is not well sustained. Occasionally there occurs more or less linear decline of action-potentials for several seconds, followed by further irregular declines or by irregular increases. In figure 3 is an illustration of linear progressive relaxation, showing that this can occur with diagram-like precision. Such precision harmonizes with the view held by many investigators that action-potentials are a definite function of the physicochemical process underlying normal muscular contraction.

In summary, 13 out of 14 students fail to attain complete relaxation in the arm muscles, following bending, within 1.0 second. At this interval, on the average, V_m has fallen to about 3.0 microvolts. In some instances

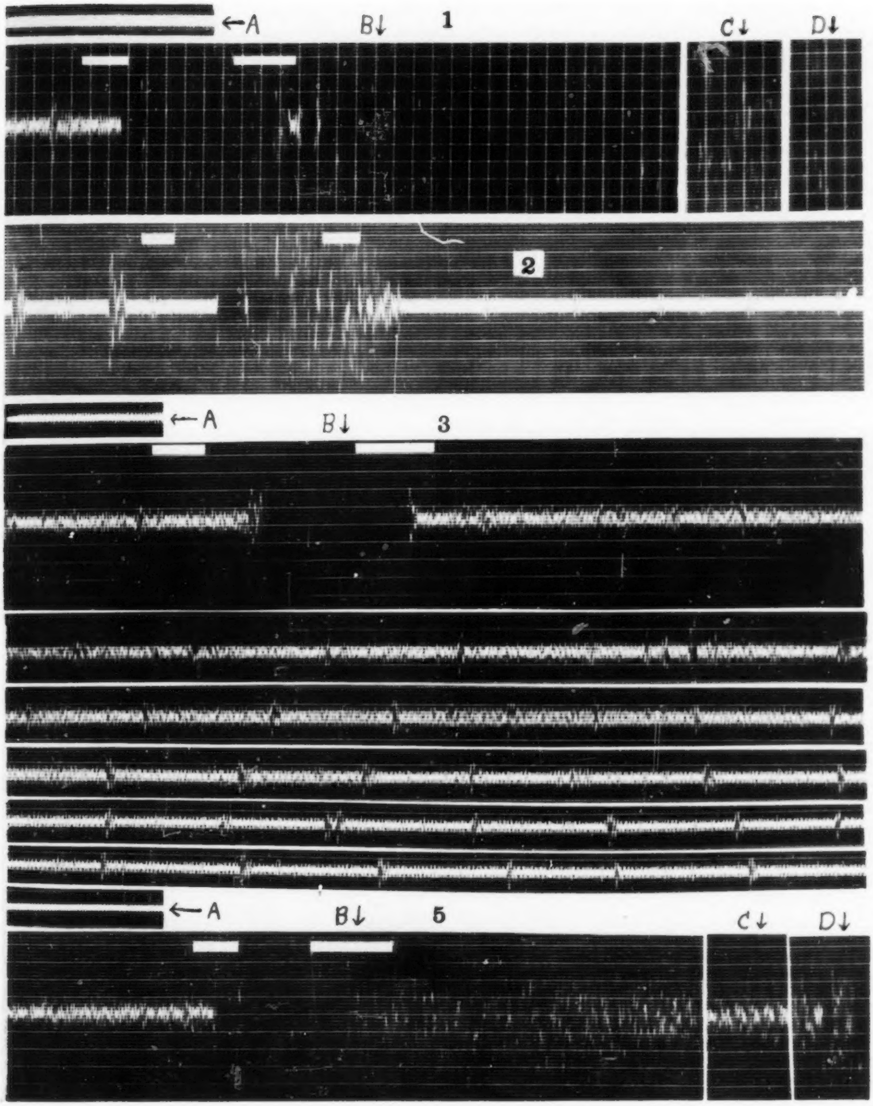
Figs. 1, 2, 3, 5. Intervals between vertical time lines = 0.2 second. This time interval applies to all records, which should be read left to right. Signal marks at the top of records indicate clicks of a telegraph key. The subject is to bend the right forearm slightly when he hears the first signal and is to relax as quickly and completely as possible when he hears the second signal.

Fig. 1. Distance between horizontal lines: 1 mm. = 0.4 microvolt. A. Short circuit across input terminals of amplifier. B. Subject S. D., a student not trained to relax. Upon the first signal, he bends the right forearm slightly: action-potentials reach relatively high voltage, so that the excursions of the shadow pass beyond the limits of the photograph. About 0.4 second after the signal to relax, the excursions diminish, but for less than 0.2 second, after which they again pass off the photograph. This failure to relax continues through C and D, although the intervals between B and C and C and D are 30 seconds.

Fig. 2. (Excursions on short circuit same as in fig. 1, A.) Subject T. N., another untrained student. Note action-potentials, indicating failure to relax as directed, prior to first signal. (In trained subjects, action-potentials are then absent as a rule.) About 0.8 second after the second signal, he evidently relaxes completely, no action-potentials being noted except periodic ones due to arterial pulsation.

Fig. 3. A. Excursions on short circuit. B. Illustrating linear progressive relaxation in an untrained student. The original record was cut into six portions: the right end of the first portion is continuous with the left end of the second portion and so on. Other conditions same as in figures 1 and 2.

Fig. 5. 1.4 mm. = 1 microvolt. A. Short-circuit. B. Failure to relax in a highly neurotic subject. Note higher voltages of action-potentials. Interval between B and C = 46 seconds; between C and D = 105 seconds.



Figs. 1, 2, 3, 5

failure to relax is prolonged for several minutes or more. Frequently the students fail to maintain complete relaxation while awaiting the first signal.

Patients before training to relax. The records show that neurotic subjects often exhibit striking failure to relax upon signal. While they await the signal to bend the arm, they often show marked action-potentials. An illustration appears in figure 5, where even 2 minutes after the signal to relax, the potentials exceed 10 microvolts.

Of 16 patients here considered no more than four relax upon signal, even if we disregard the first test recorded for each subject. At one second

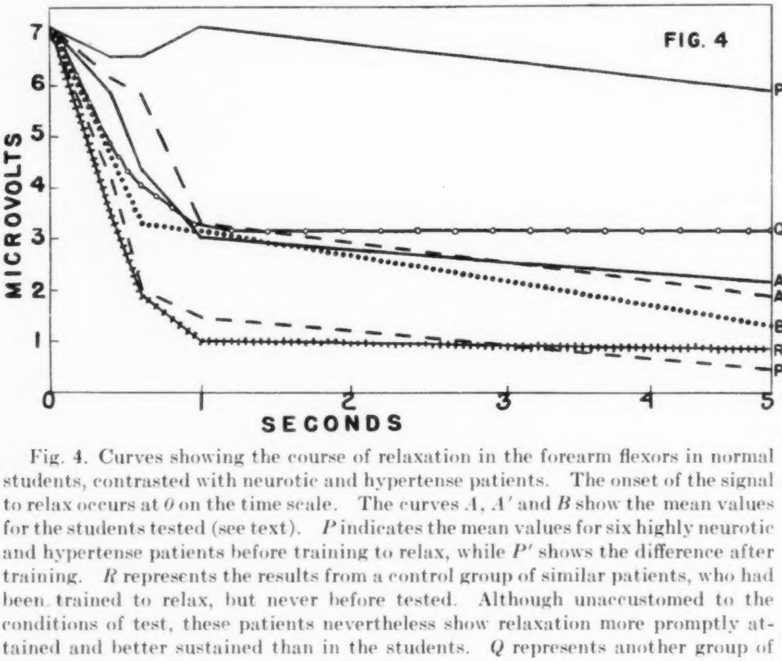


Fig. 4. Curves showing the course of relaxation in the forearm flexors in normal students, contrasted with neurotic and hypertense patients. The onset of the signal to relax occurs at *0* on the time scale. The curves *A*, *A′* and *B* show the mean values for the students tested (see text). *P* indicates the mean values for six highly neurotic and hypertense patients before training to relax, while *P′* shows the difference after training. *R* represents the results from a control group of similar patients, who had been trained to relax, but never before tested. Although unaccustomed to the conditions of test, these patients nevertheless show relaxation more promptly attained and better sustained than in the students. *Q* represents another group of patients (see text).

after signal and thereafter, the microvoltage for these four subjects generally is less than 1.4. (Three of these 4 subjects likewise showed lower mean V_m per half-hour during general rest than did the other 12 subjects in this group.)

In figure 4, the curves *P* and *Q* represent these 16 patients, divided into two groups (6 and 10). The members of the smaller group, *p*, (see loc. cit., figure 3) exhibited very marked restlessness before training to relax, as judged by clinical observation and by electrical measurements. At any instant during the five seconds after the signal to relax, the action-poten-

tials for this group, p, greatly exceed the action-potentials for either group of students. Likewise during the tests on prolonged rest the action-potentials for this group generally exceeded those for either group of students. On the other hand group q shows moderately higher action-potentials than the groups of students, beginning approximately 1.5 second after the signal to relax. It seems warranted to conclude that continuing or intermittent high action-potentials (e.g., 5 microvolts, with the present set-up) characterize the failure to relax in certain neurotic or hypertense subjects; but if such action-potentials are moderate, the difference from normal states is not sufficiently great to be distinctive.

Patients after training to relax. Following a period of training which varies for each subject, the results for group p are presented in figure 4 as the curve P'. At the end of one second, the microvoltage for each subject is less than 1.5, which is less than half the values for the students at that time, and considerably less than one-fourth of the microvoltage present at that interval when tested before training.

As previously, it is necessary to consider what part habituation to the conditions of the test may play in this reduction of relaxation times. Accordingly, we employ another set of patients (5) (see figure 5, loc. cit.), who have been trained to relax but without electrical testing hitherto. These patients, also, exhibited very marked restlessness before training to relax, as judged by clinical observation. The curve for this group, R, runs very closely to that for the other patients previously mentioned, after training, the average microvoltage at the end of one second being approximately 1.0. This is evidence that the more prompt relaxation observed in patients after training is due not to repetition of tests, but to training.

Slight contractions. During activities such as imagining or recalling, as previously stated (loc. cit.), slight but specific muscle contractions commonly take place which can be relaxed directly by the trained subject but which in both trained and untrained subjects evidently subside when the particular mental activity ceases. These experiments are here repeated because improved apparatus makes possible a more satisfactory determination of the moment when the action-potentials disappear completely. Subjects are again employed who upon instruction can maintain complete relaxation. Otherwise voltage changes due to failure to relax occur irregularly in the record and render it difficult or impossible to identify those changes which mark the act of imagination or recollection. The instruction is to "imagine bending the right arm" or to "imagine lifting a ten-pound weight in the right hand" at the first click of a telegraph key and to relax any muscular tensions present at a second like signal. An alternative instruction is to cease to imagine at the second signal.

The results for the various subjects are on the whole similar to those described in this article following the instruction to bend the arm. Sub-

jects showing marked restlessness as judged by clinical observations and by electrical measurements during prolonged attempted rest as a group show longer relaxation times than the presumably "normal" students; but after training the relaxation times are significantly shortened.

SUMMARY AND CONCLUSIONS

Of 14 students not trained to relax, 13 fail upon signal to relax the forearm flexors, following flexion, within 1.0 second. At this interval, on the average, V_m has fallen to about 3.0 microvolts. In some instances failure to relax is prolonged for several minutes or more. Prolonged relaxation may be linear in its progress. Frequently the students fail to maintain complete relaxation while awaiting the signal to bend the arm.

Certain patients are selected for test who before training to relax exhibit very marked restlessness as judged by clinical observation and as confirmed by electrical measurements of prolonged rest, showing relatively high microvoltages. These patients before training exhibit failure to relax upon signal much more striking than any of the students tested, with one possible exception. Certain other patients definitely neurotic or hypertense at times, according to clinical observation, nevertheless give curves like those of some of the students. After training, the patients all relax while awaiting the signal and they relax more promptly and completely as a rule than do the untrained students ($V_m = 1.5\mu v$ at the end of a second). (No practise at relaxing upon signal was included in the course of training.) This affords further confirmation that relaxation can be cultivated in man. Similar confirmatory evidence of shortened relaxation-times in the trained is found upon testing the slight muscular contractions characterizing certain mental activities.

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COLLATERAL RESPIRATION

The Chemical Composition and Volume of the Collaterally Respired Gases

G. E. LINDSKOG[1] and H. H. BRADSHAW[2]

From the Surgical Laboratories of the Massachusetts General Hospital and the Department of Physiology, School of Public Health, Harvard University

Received for publication February 24, 1934

Van Allen and his co-workers (1) in 1930 advanced experimental and clinical evidence to show that the lung lobules of man and certain animals are not isolated units or end structures from the standpoint of their ventilation. They demonstrated that a lung lobule, the bronchus of which had become obstructed, could obtain air from adjacent, freely ventilated lobules. Subsequent work by the same investigators (2), (3), (4) pointed out the significance of this phenomenon, which they termed collateral respiration, in the physiology and pathology of the lung; for example, its rôle in minimizing the incidence of lobular atelectasis and obstructive emphysema, the assistance which it renders to the cough mechanism, and the rôle it plays in the reinflation of atelectatic lobules.

Up to the present time no study has been made of the chemical composition of collateral air, that is, the air which passes collaterally to and from an obstructed lobule spontaneously in respiration. Neither have volumetric studies been made to determine the relative efficiency of collateral respiration as compared with direct respiration and to ascertain the quantitative effects of such factors as depth of respiration and cough. The present paper is a report of experimental work on these general problems.

Part I. The chemical composition of the gases respired by collateral channels following bronchial obstruction as compared with: 1, the composition of gases respired by unobstructed parts of the lung; 2, the gases imprisoned in an obstructed lobe; 3, the blood gases. Experimental methods and materials. Healthy dogs varying from 20 to 30 kgm. in weight were used exclusively. Each animal was anesthetized by the intraperitoneal injection of sodium barbital, in dosages of 0.33 gram per kilogram of body weight. The drug was dissolved in

[1] Fellow of the National Research Council.

[2] We wish to express our appreciation to Dr. Albert R. Behnke, U. S. N., Research Fellow in Physiology at Harvard University, for his assistance in analyzing the blood samples.

approximately 50 cc. of warm physiologic saline solution. When the animal was deeply anesthetized, usually in the course of one hour, a mid-line tracheotomy was performed and a cannula of glass tied into the tracheal opening. This cannula had the approximate calibre of the trachea itself and was equipped with two side openings of slightly smaller diameter. To compensate for the decrease which the tracheotomy effected on the natural dead space, a wide rubber tube 20 cm. in length was attached to one side arm of the tracheal cannula (fig. 1, A); the other side arm and the

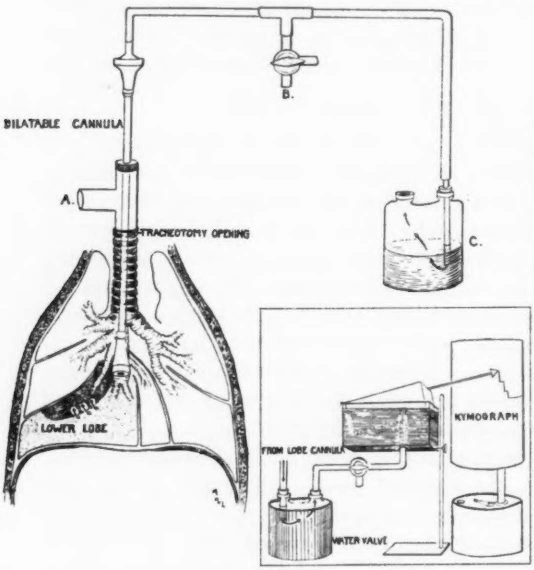

Fig. 1

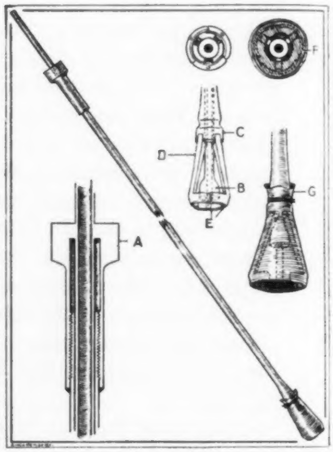

Fig. 2

Fig. 1. Diagrammatic sketch of apparatus used for collection of air from obstructed lung lobules. Insert: Arrangement for volume measurements of transpired air.

Fig. 2. Diagrammatic sketch of dilatable bronchial cannula.

middle outlet through which a bronchial cannula passed were closed with plasticine.

A long and slender metal bronchial cannula (fig. 2) with a mechanically dilatable tip (designed by Dr. C. M. Van Allen, and adequately described in a previous communication (5)) was then introduced through the tracheal cannula well into a tertiary bronchus of the right or left lower lobe, and dilated in position to give a firm and air-tight occlusion of the stem bronchus below the origin of the two or three proximal side bronchi. The lung cannula was then connected at its outer end with a glass tube of similar

bore, which opened at a depth of 0.5 cm. under a surface of acidulated water. Between the cannula and this water valve was interposed a glass T-tube, the side arm of which communicated with a three-way stopcock. All connections were made with heavy gum tubing and the usual precautions against leaks in gasometric work were observed. During each expiration, when the three-way stopcock was closed, air bubbles were seen to pass from the submerged tube of the water valve to the outside. With each inspiration, the water seal rose in the glass tube, preventing any access of air to the occluded lobules, except that which might come through collateral channels from adjacent free lobules.

Sampling of the transpired gases was accomplished by attaching a mercury sampling tube of 50 cc. capacity to the three-way stopcock (fig. 1, B), opening the stopcock, rinsing the connections with two 5 or 10 cc. quantities of gas, and then withdrawing gas by a *very slight* negative pressure at a rate of approximately 20 cc. in 10 minutes. This rate was insufficient to disturb the rhythmic expiratory discharge of air through the water valve, or to raise the level of water in the tube during inspiration beyond the usual slightly negative level.

In certain experiments in which samples of the mixed expired air from the entire lung field were collected simultaneously with the transpired collateral air, an automatic air sampling device was attached to the tracheal outlet (fig. 1, A). This device permitted a free inspiration for the animal through a Tissot valve, but expiration only through a Krogh box-valve, which trapped the last few cubic centimeters of each expiration. A fraction of this air, which will be referred to as the *terminal expired air*, was drawn into a collecting tube from the box-valve during each succeeding inspiration. Analyses of gas samples were made for oxygen, carbon dioxide and residual nitrogen in a Haldane-Henderson apparatus. The results have been expressed in percentages (dry volume) and in tension millimeters, according to the formula: Tension = (Barometric Pressure − Vapor Tension) × per cent gas.

In certain experiments in which the carbon dioxide tensions of the blood were determined, samples were drawn during the course of the corresponding air collection by introducing a needle into the common carotid artery. The blood was removed in an oiled syringe without exposure to air, and preserved with potassium oxalate over mercury until analyzed in a Van Slyke apparatus. At the same time, two blood samples were taken aerobically for equilibration in a tonometer against known tensions of carbon dioxide (near 40 and 70 mm. respectively). From the observed carbon dioxide content of the equilibrated specimens, the carbon dioxide dissociation curve of the oxygenated dog's blood was plotted, and the carbon dioxide dissociation curve for the corresponding reduced blood calculated. Having determined by direct analysis the percentage carbon dioxide con-

tent of the unknown arterial blood sample and its percentage saturation with oxygen, its carbon dioxide tension was determined from the dissociation curves.

In three dogs experiments were conducted to compare the effects on the respiratory gases of bronchial obstruction with and without collateral respiration. For this purpose the bronchial cannula was dilated at such a level that it completely obstructed the left lower lobe. Making certain that no collateral respiration was obtained, as evidenced by the absence of passage of air through the water valve (fig. 1, C), the cannula was occluded above this outlet, and after 20 minutes the contained air was sampled rapidly and analyzed. The bronchial cannula was then collapsed, reintroduced into a lower (intralobar) position, and dilated. The collateral passage of air was then observed through the valve (fig. 1, C) with each expiration: the cannula was occluded above this valve outlet, and the contained air sampled after a similar period of 20 minutes.

TABLE 1

Comparison of collateral transpired air and mixed expired air samples simultaneously collected

DOG NO.	TIME OF COLLECTION		SAMPLE AIR	CARBON DIOXIDE		OXYGEN	
	Began	Ended		Per cent	Tension	Per cent	Tension
					mm.		*mm.*
1	5:00	5:15	Collateral	5.09	36	12.63	89
			Expired	3.99	28	15.25	107
2	3:38	3:48	Collateral	4.91	34	13.91	98
			Expired	3.05	21	16.47	116
3	11:10	11:22	Collateral	5.43	38	10.69	75
			Expired	5.15	36	12.56	88
4	12:21	12:33	Collateral	5.27	37	12.89	90
			Expired	4.18	29	14.63	102

After all experiments the animal was sacrificed with an intravenous injection of chloroform: the chest was autopsied to check the position and tightness of the occluding cannula and to rule out the presence of gross lung pathology.

RESULTS. 1. *The simultaneous collection of collateral air and terminally expired air samples.* Seventeen different determinations were made on four dogs: the results were in uniform agreement. The carbon dioxide tension of the collateral air sample was always higher than that of the terminal expired sample (the average difference was 6.7 mm.). The oxygen tension

of the collateral sample was always lower than that of the simultaneously collected terminal expired air (the average difference was 18.3 mm.). Four typical experiments are tabulated in table 1.

The carbon dioxide and oxygen tensions for the samples of collateral air were observed to be within ranges which suggested a possible relationship to the true alveolar tensions. Since there is no method of obtaining reliable alveolar air samples directly in anesthetized animals, it was decided to determine this point indirectly from the arterial blood carbon dioxide tensions.

2. *Comparison of carbon dioxide tensions in the collateral air samples and the arterial blood.* This series consisted of nine determinations on three different dogs. Table 2 summarizes the results.

TABLE 2

DOG NO.	TIME OF SAMPLING		CO_2 TENSION	
	Collateral air	Arterial blood	Collateral air	Arterial blood
			mm.	*mm.*
4	11:10–11:25	11:22	48	49
	12:21–12:33	12:28	37	43
	1:47– 1:58	1:52	35	41
5	11:18–11:29	11:27	34	51
	1:21– 1:28	1:25	35	44
	2:34– 2:38	2:35	39	39
6	11:37–11:41	11:38	40	43
	11:47–11:54		42	
	1:35– 1:40	1:37	37	43
	1:47– 1:51		40	

It was observed that in three instances the carbon dioxide tensions of collateral air and arterial blood were nearly or quite the same; in the remaining six, the collateral air carbon dioxide tension was below the blood carbon dioxide tension.

In two animals the method of collecting the collateral air samples was varied somewhat. The bronchial cannula was occluded above the water valve (fig. 1 C) so that it permitted no egress of air during expiration. After 15 or 20 minutes the contained air was sampled rapidly to the extent of approximately 20 cc. and an arterial blood sample was taken at the same time. The results are presented in table 3.

These four determinations show similar findings to those observed with the continuous sampling technic (table 2).

A series of determinations was made contrasting the two methods of sampling: continuous sampling with the expiratory valve functioning, and

instantaneous sampling 15 or 20 minutes after occlusion of the valve. The results are presented in table 4.

The method of instantaneous sampling gave uniformly higher carbon dioxide tensions and lower oxygen tensions than the continuous method.

3. *A comparison of the effects on the respiratory gases of bronchial obstruction with and without collateral respiration.* In three dogs successive determinations were made of the respiratory gases imprisoned behind a complete lobar obstruction and a complete lobular obstruction, as de-

TABLE 3

DOG NO.	TIME		CO_2 TENSIONS	
	Valve occluded	Sampling	Collateral air	Arterial blood
			mm.	*mm.*
7	11:03	11:25	52	52
8	11:04	11:24	36	47
	1:22	1:42	35	43
	2:32	3:09	36	36

TABLE 4

DOG NO.	METHOD OF SAMPLING	TIME	CARBON DIOXIDE		OXYGEN	
			Per cent	Tension	Per cent	Tension
				mm.		*mm.*
9	Continuous	10:30–10:40	4.45	32	14.36	103
	Instantaneous	11:18	5.33	38	13.85	99
	Continuous	11:26–11:35	4.37	31	15.11	108
10	Instantaneous	2:52	5.99	42	10.17	72
	Continuous	2:58– 3:08	5.62	39	11.17	79
8	Continuous	12:14–12:31	4.58	33	14.14	101
	Instantaneous	1:19	5.28	38	12.52	89

scribed under Methods. In the former situation, of course, no collateral respiration could occur. The results are summarized in table 5.

The effect of a total lobar obstruction was to cause a rapid fall in the oxygen tension, and rise in the carbon dioxide tension of the imprisoned air, to the mixed venous blood tensions. In the lobular obstruction, on the contrary, where collateral respiration was in effect, the oxygen tensions were higher and the carbon dioxide tensions lower, at the approximate levels for arterial blood (cf. table 2).

PART II. VOLUMETRIC STUDIES: THE RATE OF PASSAGE OF AIR BY THE

COLLATERAL ROUTE. METHODS AND MATERIALS. The preparation of the animal and the introduction of the bronchial cannula were identical to the procedure described in Part I. The lung cannula opened under a water seal, which was contained in a small airtight bottle. The bottle was connected directly with a delicately balanced Krogh spirometer of 100 cc. capacity. The transpired gases passed through the water valve into the bottle and on into the spirometer, the movement of which was recorded by a light writing arm on a smoked kymograph drum (fig. 1, insert). The volume of the spirometer was calibrated before each experiment by introducing measured amounts of air progressively; the speed of the kymograph clock was likewise calibrated.

In order to determine the tidal volume of the free portion of the lung synchronously with the measurement of the collaterally transpired air, the

TABLE 5

DOG NO.	TYPE OF BLOCK	CARBON DIOXIDE		OXYGEN		VENOUS BLOOD CARBON DIOXIDE
		Per cent	Tension	Per cent	Tension	
			mm.		*mm.*	*mm.*
4	Lobar block	6.98	49	6.55	46	50
7	Lobar block	7.82	56	6.60	47	
	Intralobar block	7.21	51	10.57	76	
10	Lobar block	6.96	49	6.88	48	
	Intralobar block	5.99	42	10.17	72	
11	Lobar block	6.23	44	5.56	40	
	Intralobar block	5.58	39	10.43	74	

open arm of the tracheal cannula (fig. 1 A) was connected with a closed circuit consisting of a Roth-Benedict recording spirometer, containing oxygen, with Tissot valves to direct the flow of air, and a soda-lime reservoir to eliminate carbon dioxide.

In three dogs groups of determinations were made during the course of an hour, recording the general tidal volume and the volume of collateral air synchronously. The determination of relative efficiency in the collateral channels was reached by dividing the collateral volume per respiration by the tidal volume per respiration. This result was then multiplied by 10, since the obstructed lobules represented roughly one-half of the lower lobe, which in turn constitutes about one-fifth of the total lung field.

The effect of increasing respiratory depth upon the volume of collateral respiration in an obstructed lung field was determined by allowing an animal prepared as described above to rebreathe from the spirometer filled

with oxygen, the soda-lime having been removed from its reservoir to allow accumulation of carbon dioxide progressively during the experiment.

The effect of expiratory resistance and positive pressure upon the volume of collateral respiration was studied by adding graduated weights (100, 200, 300 gm.) to the bell of the Roth-Benedict spirometer. The effect of this manoeuvre was to introduce slight positive pressure to the whole lung field.

TABLE 6

DOG NO.	COLLATERAL VOLUME			TIDAL VOLUME	EFFICIENCY
	Cc. per min.	Resp. rate	Cc. per resp.		
				cc.	*per cent*
3	60	24	2.5	289	9
	83	28	2.9	336	9
	89	30	2.9	352	8
	93	36	2.6	342	8
	106	45	2.4	315	8
12	36	19	1.9	186	10
	50	21	2.4	228	10
	33	19	1.7	186	9
8	106	32	3.3	259	13
	112	32	3.5	290	12

TABLE 7

Dog 11

ELAPSED TIME	COLLATERAL VOLUME			TIDAL VOLUME	EFFICIENCY
	Cc. per min.	Resp. rate	Cc. per. resp.		
minutes				*cc.*	*per cent*
0	37	25	1.5	207	7
1	53	25	2.1	269	8
2	73	30	2.4	300	8
3½	90	34	2.6	290	9
Spirometer washed with oxygen					
0	24	24	1.0	197	5
1	70	35	2.0	290	7
2	92	40	2.3	321	7

Carbon dioxide in spirometer sample at conclusion: 8.9 per cent.

RESULTS. 1. *The volume of collaterally respired air and the relative efficiency of the collateral route when compared with normal bronchial ventilation.*

TABLE 8

DOG NO.	WEIGHT ON SPIROMETER	COLLATERAL VOLUME			TIDAL VOLUME	EFFICIENCY
		Cc. per min.	Resp. rate	Cc. per resp.		
	grams				*cc.*	*per cent*
12	0	33	19	1.7	186	9
	100	55	18	3.1	248	12
	200	67	21	3.2	269	12
	300	72	20	3.6	248	15
8	0	112	32	3.5	290	12
	100	184	40	4.6	331	14
11	0	24	11	2.1	248	8
	100	43	15	2.8	290	10
	200	76	17	4.5	300	15

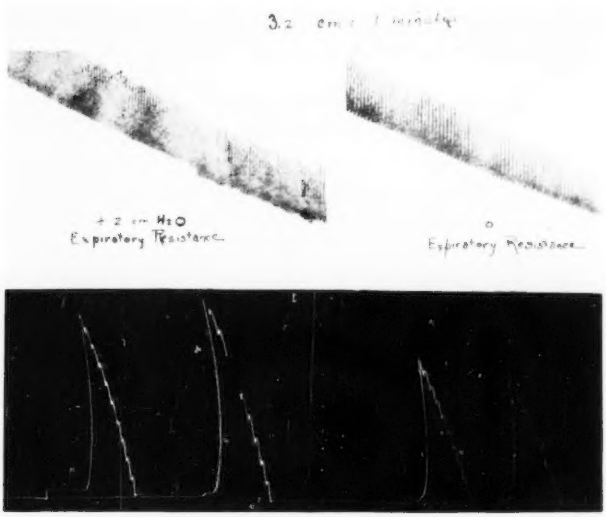

Fig. 3. Record showing the augmentatory effect of expiratory resistance on the collateral passage of air. Dog 8. Table 8. (Above kymograph record of the total pulmonary ventilation. Below: spirometer record of the collateral ventilation in the obstructed lobules.)

Experiments were conducted upon three dogs with the results presented in table 6.

In the following experiments the relation between increasing depth of respiration due to carbon dioxide accumulation and the collateral passage of air was observed (table 7).

These experiments showed that the volume of collateral respiration tended to vary directly with the depth of respiration, while the relative efficiency was fairly stable in a given animal.

2. *The effect of positive pressure on the rate of collateral respiration.* The addition of small graduated weights to the bell of the Roth-Benedict spirometer gave the following results in three experiments (table 8).

These experiments demonstrated that the addition of slight resistance to respiration caused both an actual and a relative increase in the volume of collateral respiration. Figure 3 presents a typical record from one of these experiments.

Discussion and conclusions. These results are to a certain point in agreement with those of Loewy and von Schrötter (6) and Coryllos (7) who found that when air is completely imprisoned in a lung field, it very rapidly reaches a composition in which the tensions of carbon dioxide and oxygen are those of the mixed venous blood returning to the lungs. The first experimenters used human subjects, the second repeated the observations on dogs, both groups using dilatable balloon-tipped bronchial cannulae. The type of instrumentation must have resulted in a complete obstruction of one or more lobes.

There is, however, a striking and important difference in the case of obstruction to a *portion* of a lobe when collateral respiration comes into play, for the carbon dioxide tensions, as we have shown, do not rise above the carbon dioxide tension for mixed arterial blood, and the oxygen tension falls to a level approximately that in alveolar air. Of course, this is best explained by the collateral ventilation.

As has been shown in Part II, the collateral pathways may be at least ten per cent efficient as compared with the normal bronchial route. That the actual efficiency is greater than this figure may be safely assumed since the expiratory water valve mechanism probably does not collect all of the collateral air which has passed to the obstructed area during the preceding inspiration. Some of this air probably departs by the same collateral channels before the expiratory collapse raises the resistance in the collateral channels to a point above that in the cannula-water valve route of exit.

The importance of collateral respiration to the physiology of respiration will be immediately apparent upon consulting the normal oxygen dissociation curve for dog or human blood. At the tensions of oxygen which obtain in lobar obstruction without the possibility of collateral respiration (for example, 47 mm.), the blood leaving the obstructed area remains to a considerable extent unsaturated (75 per cent saturation): the more venous the blood as it returns to the lungs, the more marked the unsaturation; the oxygen tension in the mixed arterial blood will therefore be significantly less than normal. At the tensions of oxygen which obtain in the gas behind a lobular obstruction, where collateral respiration is active (let us

say 80 mm.), the blood leaving the obstructed area shows a saturation of 90 to 95 per cent, and the degree of unsaturation of the mixed arterial blood is minimal. For example, in dog 7 the percentage saturations of the three arterial blood samples were 94.54, 94.93, and 98.25 respectively.

This conserving effect of collateral respiration on the oxygen of the venous blood in an obstructed lobule helps us to understand the observed fact that patients may have extensive exudative lesions in the lungs and still show little or no unsaturation in the mixed arterial blood.

The volumetric data presented show that the volume of collateral respiration bears a fairly constant relationship to the tidal flow, within certain limits, confirming the observation repeatedly made that very shallow breathing may abolish the phenomenon entirely, at least in so far as it can be measured by the water valve technic.

The decided increase in the volume of collateral ventilation with slight positive pressure in the lung fields is presumably dependent on the same mechanism as the increase in the collateral volume during deep breathing. In each case there is an increase in the effective differential pressure which is responsible for the collateral passage of air from the freely ventilated lobules to the occluded lobules during the inspiratory phase.

SUMMARY

Using dogs anesthetized with sodium barbital, the gases respired collaterally by obstructed lung lobules have been collected, measured, and analyzed.

1. In lobar obstruction (where collateral respiration does not occur) the gas in the obstructed area rapidly comes into equilibrium with the venous blood gas tensions: whereas, in lobular obstruction, where collateral respiration is effective, the gas in the obstructed area never shows a carbon dioxide tension above the arterial carbon dioxide level, and shows a correspondingly higher oxygen tension. The importance of this phenomenon in the respiratory function is discussed.

2. An obstructed lung lobule may breathe through collateral respiratory channels at least ten per cent as much air as it would normally through direct bronchial channels.

3. The volume-flow of collateral air varies directly and proportionately within certain limits to the depth of respiration (tidal volume): when positive pressure breathing is instituted, this causes the volume flow of collateral air to increase out of proportion to the depth of respiration.

4. Collateral respiration appears to have economic significance in bronchial obstruction, in that it permits an obstructed lung field to carry on a considerable ventilation which may be sufficient, indeed, to oxygenate the venous blood passing through the vessels in the obstructed area.

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THE EFFECT ON THE WEIGHT OF THE OFFSPRING OF ADMINISTRATION OF ANTUITRIN G TO THE PREGNANT RAT

L. W. SONTAG AND PAUL L. MUNSON

From the Samuel S. Fels Fund, Antioch College, Yellow Springs, Ohio

Received for publication March 8, 1934

The purpose of this investigation was to determine what effect, if any, is produced upon rat feti when excesses of the active growth principle of the anterior pituitary gland are introduced into the maternal circulation. Evans and Long demonstrated the existence of the growth hormone in 1921 and showed that when injected into adult rats it produced marked weight increases. Evans and Simpson (1931) have shown that adult females were more affected than males. Teel (1926) has reported that injections into pregnant rats of an alkaline extract of beef pituitary somewhat cruder than is now available produced heavier pups. His results were not subjected to statistical analysis, however. Engelbach (1932 a, b) has advocated the use of Antuitrin G (Parke Davis) or Growth Hormone (Squibb) during human pregnancy where there is a history of hypopituitarism in the family. He states that such a procedure would have no effect upon the course of pregnancy. Our work with Antuitrin G confirms much of Teel's work and throws grave doubt upon the validity of Engelbach's statement concerning the effect upon the course of pregnancy.

Our experimental group consisted of individuals from six litters of mature female rats, and the control group the remainder of the colony on a normal regimen. All were descendants, aged four months or more, removed by one to three generations from a single litter of the Wistar Experimental Colony strain of albino rats.

In order to avoid delay of implantation, subcutaneous injections of ½ cc. daily of Antuitrin G[1] were begun on the eighth day following insemination (determined by discovery of sperms in the vaginal smear). This dose was increased to 1 cc. daily on the tenth day and was continued through the twenty-second. Controls were kept under the same conditions and received the same diet (Sherman diet B, modified by replacement of one-half the NaCl by $CaCO_3$) but received no injections. Daily weight and vaginal smear records were kept in a number of subjects, both experimental and control.

All pups were weighed individually on the morning following delivery,

[1] Kindly furnished by Dr. Oliver Kamm, Parke, Davis & Company.

except in a few of the first litters, which were weighed collectively. All those not killed for chemical analysis were then weighed at intervals of five days until aged fifty days.

Results. 1. *General.* Table 1 gives the number of stillbirths and the distribution between the sexes of the 104 experimental pups and the 149 control pups. The proportion of stillbirths was about three times greater in the experimental than in the control group, and within the experimental group the mortality of the males was much higher than that of the females.

TABLE 1

Sex and stillbirth incidence

EXPERIMENTAL						CONTROL					
104						149					
Male		Female		Unknown		Male		Female		Unknown	
44		52		8		58		86		5	
Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead
21	23	42	10	2	6	51	7	78	8	0	5

TABLE 2

Means and variation of body weights with number per litter

AGE	NO. CASES		AVERAGE WEIGHT				COEFFICIENT OF VARIATION				"r" OF WT. AND NO./LITTER	NO. / LITTER	
	Exp.	Con.	Exper.	Control	Difference	*	Exper.	Control	Difference	*		Exp.	Con.
			g.	g.	g.		g.	g.	g.				
Birth	78	114	5.85 ±0.04	5.45 ±0.04	0.40 ±0.06	6.7	8.95 ±0.49	11.23 ±0.51	2.28 ±0.71	3.2	−0.37 ±0.04	7.5	7.8
5 days	34	65	11.71 0.15	10.78 ±0.18	0.93 ±0.24	3.9	11.07 ±0.91	20.41 ±1.26	9.34 ±1.55	6.0	−0.35 ±0.05	5.9	6.0
10 days	34	64	20.81 ±0.25	19.13 ±0.34	1.68 ±0.43	3.9	10.51 ±0.87	21.24 ±1.32	10.73 ±1.58	6.8	−0.49 ±0.04	5.9	5.9
20 days	34	64	38.4 ±0.59	37.1 ±0.59	1.3 ±0.83	1.6	13.33 ±1.11	18.73 ±1.[illegible]5	5.40 ±1.60	3.4	−0.59 ±0.04	5.9	5.9
25 days	34	63	51.7 ±0.66	50.0 ±0.69	1.7 ±0.96	1.8	11.02 ±0.91	16.25 ±1.00	5.23 ±1.35	3.9	−0.44 ±0.05	5.9	5.8

* Diff./P.E. Diff.

2. *Increase in birth weight of pups.* Analysis of all the individual birth weights available showed a reliable increase in birth weight amounting to 0.4 gram (7.3 per cent) occasioned by the prenatal influence of Antuitrin G

(see table 2). (Correction for a preponderance of females over males in the control group relative to the experimental group raised the control mean only 0.01 g.)

Out of a total of twelve experimental litters only four litters were born after a gestation period of normal duration. Parturition was delayed one day or more in the other eight litters. The mean birth weights of these experimental litters in which parturition was delayed were not, however, significantly higher than those of the experimental litters whose gestation was of normal duration. (Birth weight of delayed experimental litters—birth weight of normal experimental litters = $5.88 - 5.81 = 0.07 \pm 0.073$ g.)

While experimental and control mothers varied considerably in age and weight, no significant correlation was found between birth weight of pup and age or weight of mother. There was a negative correlation between

TABLE 3

Weights of experimental and control litters from the same mothers

DAM	EXP. LITTER			CON. LITTER			EXP. MINUS CON.
	No.	No. pups	Mean birth wt.	No.	No. pups	Mean birth wt.	
			g.			g.	g.
5	27	9	6.02	13	12	5.11	+0.91
6	18	12	5.58	26	10	4.17	+1.41
7	28	4+	5.60	14	9	5.78	−0.18
8	19	7	6.06	30	8	5.57	+0.49
9	22	9	5.72	23	7	5.89	−0.17
10	25	9	5.95	21	9	5.36	+0.59

$M_{exp.} = 5.82 \qquad M_{con.} = 5.26$

$M_{exp.} - M_{con.} = 0.56$

birth weight of pup and the number born in the litter ($r = -0.37 \pm 0.04$). However, the size of the litters of the control group at birth was approximately the same as that of the experimental group, so it was not necessary to consider this factor.

As a somewhat different approach to the problem, six females from two litters were each allowed to produce two litters. Three of the females were injected with Antuitrin G d ing their first pregnancy and received no injections during the second, which occurred at least two months later. The reverse procedure was followed in the other three cases. Results are shown in table 3. The number per litter of the experimental and control groups was approximately the same. Four out of the six animals bore appreciably heavier experimental than control litters, while both litters were approximately of the same weight in the other two females. (One of these two animals received injections during her first pregnancy, the

other one during the second.) These figures suggest that while Antuitrin G is effective in accelerating growth in utero when administered during pregnancy to the mother, there is much individual variability in response to it.

3. *Variability in birth weight of pups.* The birth weights of the experimental group varied somewhat less than those of the control group. (See table 2.)

4. *Effect on sex differences in birth weight.* The slight weight advantage of control males over control females (Difference = 0.14 ± 0.078 g.; Diff. / P.E. of Diff. = 1.8) was considerably increased in the experimental group where the mean weight of the experimental males was 0.36 ± 0.08 g. (Diff. / P. E. of Diff. = 4.5) more than that of the experimental females.

TABLE 4

Means and standard deviations of females' body weights

AGE	NO. CASES		AVERAGE WEIGHT				S. D.	
	Exp.	Con.	Exper.	Control	Difference	*	Exp.	Con.
			g.	g.	g.			
Birth	38	67	5.70±0.05	5.39±0.05	0.31±0.05	4.3	0.493	0.574
5 days	23	38	11.64±0.19	10.47±0.23	1.17±0.305	3.8	1.39	2.15
10 days	23	38	20.77±0.31	18.32±0.46	2.45±0.555	4.4	2.21	4.20
20 days	23	38	38.59±0.55	36.34±0.63	2.25±0.84	2.7	3.91	5.80
25 days	23	37	51.52±0.56	48.65±0.83	2.87±1.02	2.7	4.00	7.50
30 days	23	37	61.22±0.86	56.30±1.03	4.92±1.34	3.7	6.12	9.31
40 days	23	31	84.22±1.49	81.03±1.64	3.19±2.21	1.4	10.63	13.58
50 days	23	32	105.26±1.64	95.28±2.39	9.98±2.90	3.4	11.64	19.95

* Diff./P.E. Diff.

Both the experimental males and females were significantly heavier than the control pups of their respective sexes (Mean $_{exp♂}$ − Mean $_{con♂}$ = 0.53 ± 0.086 g., Diff./ P. E. of Diff. = 6.2; $M_{exp♀} - M_{con♀}$ = 0.31 ± 0.072 g., Diff. / P. E. of Diff. = 4.3). The experimental females were also slightly heavier than the control males ($M_{exp♀} - M_{con♂}$ = 0.17 ± 0.082 g., Diff./ P. E. of Diff. = 2.1). Evans and Simpson (1931), using rats as young as thirty days old, have shown that females, both intact and gonadectomized, are more susceptible than males to the growth-promoting effects of the growth hormone. Our work indicates that this superior female sensitivity does not apply to the prenatal effects of the hormone.

Taking the sexes separately, we found variability of both males and females less in the experimental group than in the controls ($V_{con♂} - V_{exp♂}$ = 2.85 ± 1.06 g., Diff. / P. E. of Diff. = 2.7; $V_{con♀} - V_{exp♀}$ = 2.00 ± 0.92 g., Diff. / P. E. of Diff. = 2.2).

5. *Stability of prenatal effects of Antuitrin G.* Because of the occurrence of a number of still births in the experimental group (table 1) and the sacrifice of a number of others for chemical analysis, it was not possible to observe the growth curve of the entire original group. Because of the correlation between weight and number of pups per litter suckling (table 2) it was necessary to eliminate this factor. This was done by selecting only those litters containing from four to seven suckling rats per litter.

Tables 2 and 4 show that, while after the first ten days of age the difference in weight between the two groups lost absolute significance, the experimental animals retained a slight advantage through 50 days of age. The weights of both sexes combined are given only to age 25 days because of the effect of the increasing sex-weight differential on the normal distribution. In addition, a larger number of females than of males was available.

The smaller variability of the experimental group, relative to the control group, became even smaller at five days of age and continued through ten days, but rose again at twenty days.

6. *Effects on the mother.* Delayed parturition in a majority of the injected mothers has been mentioned above. This must necessarily be caused either by the growth hormone itself or by some contaminating factor present in the preparation. It is not true, however, that those animals most susceptible to the "parturition-delaying" factor also bore litters which had been the most susceptible to the prenatal growth promoting properties of Antuitrin G.

Administration of Antuitrin G during pregnancy did not incapacitate the injected animal for subsequent normal reproduction.

CONCLUSION. In rats, at least, the course of pregnancy is usually affected by injections of growth hormone in the mother. It definitely influences the development of the rat fetus. Whether or not this influence justifies the use of Antuitrin G prenatally, as suggested by Engelbach, is impossible to state. It would seem, however, that such use might be attended by grave danger to the pregnancy.

SUMMARY

When Antuitrin G (Parke Davis) was administered to twelve pregnant rats in 1 cc. daily doses, the following results were noted:

1. The number of stillbirths was increased about three times over the number in the control group of sixteen pregnancies.

2. The average weight of the 104 experimental pups was 0.4 gram (7.3 per cent) higher than that of the 149 controls.

3. The period of gestation was increased in length in a majority of the litters but without a corresponding increase in birth weight of the pups over that of the remaining experimental litters.

4. No significant correlation was found between age of mother and birth

weight of pups, and a negative correlation existed between the birth weight of the pups and the size of the litter.

5. A considerable variability in individual response to the Antuitrin G was found.

6. The male pups showed greater weight increases than did the females.

7. The weight advantage of 34 surviving experimental pups over 65 controls was much lessened by the end of the fifth day of postnatal life, but the experimental group maintained a slight superiority in weight through fifty days of age.

8. The Antuitrin G injections did not incapacitate the rat mothers for subsequent reproduction.

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A FURTHER STUDY OF VASODILATORS IN SYMPATHECTOMIZED ANIMALS

A. ROSENBLEUTH and W. B. CANNON

With the occasional assistance of D. McK. Rioch, B. Cannon and M. McK. Sawyer

From the Laboratories of Physiology in the Harvard Medical School

Received for publication March 15, 1934

Reflex vasomotor dilatation and constriction may be obtained in sympathectomized vascular territories (Bayliss, 1902; Fofanow and Tschalussow, 1913; Bishop, Heinbecker and O'Leary, 1933; Tournade and Malméjac, 1933). Reflex rises and falls of blood pressure may, likewise, be obtained in completely sympathectomized animals (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1932a). These observations suggest vasodilator centers and central tracts susceptible of direct stimulation. The present report deals with the results of such direct central stimulation of vasodilators in completely sympathectomized animals. Some other related observations are included.

Method. Cats were used. Sympathectomy was performed as described by Cannon, Newton, Bright, Menkin and Moore (1929). Dial or ether anesthesia, or decerebration under ether succeeded by curarization, was employed for the acute experiments. The blood pressure was recorded from a carotid artery by means of a mercury manometer. The vagi were cut in the neck.

Results. A. *Effects of curare and of section and stimulation of the cervical spinal cord* (with the assistance of D. McK. Rioch). Injections of curare evoke a rise of blood pressure in sympathectomized cats (fig. 1). This effect was obtained under dial or ether and in decerebrated preparations. The rise is of interest when contrasted with the fall which occurs in normal animals in similar conditions.

Section of the cervical spinal cord (C_2 to C_5) after curare elicits no change or a slight transient fall of blood pressure in sympathectomized cats (fig. 2A); in normal animals this section causes a considerable rise of blood pressure succeeded by a persistent fall (fig. 2B).

Electrical stimulation of the sectioned spinal cord does not usually produce any changes of blood pressure in the curarized, sympathectomized animals. In only one instance out of six did there appear a slight fall of blood pressure; it was attended by a dilatation of the hind leg.

B. *Effects of ergotoxine* (with the assistance of Bradford Cannon). Two

sympathectomized cats, one decorticate and the other decerebrate (both, therefore, with the medulla intact and connected to the lower centers), exhibited a considerable rise of blood pressure when ergotoxine was in-

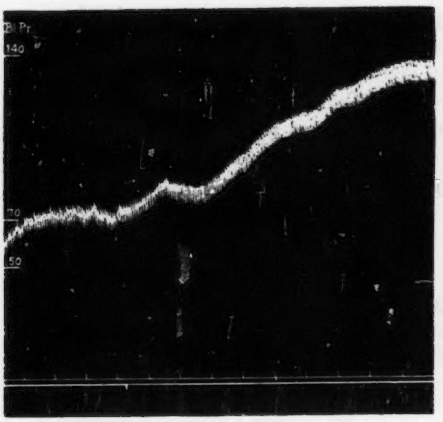

Fig. 1

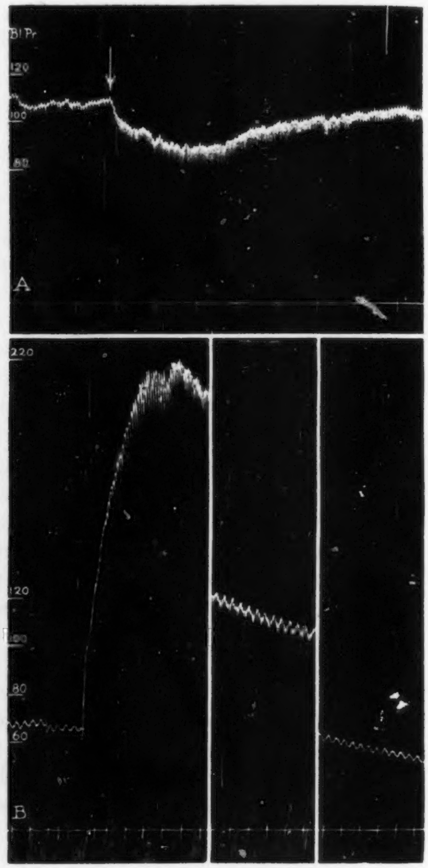

Fig. 2

Fig. 1. Completely sympathectomized; decerebrated; vagi cut. A paralyzing dose of curare was injected at the signal. In this and the other figures time is recorded at half-minute intervals.

Fig. 2 A. Completely sympathectomized; dial; curare. At the arrow the cervical spinal cord was lifted and then transected.

B. Normal; dial; curare. The sharp rise of blood pressure coincides with the lifting of the cervical spinal cord, transected immediately thereafter. The second record was taken 11 minutes, and the third one 17 minutes later.

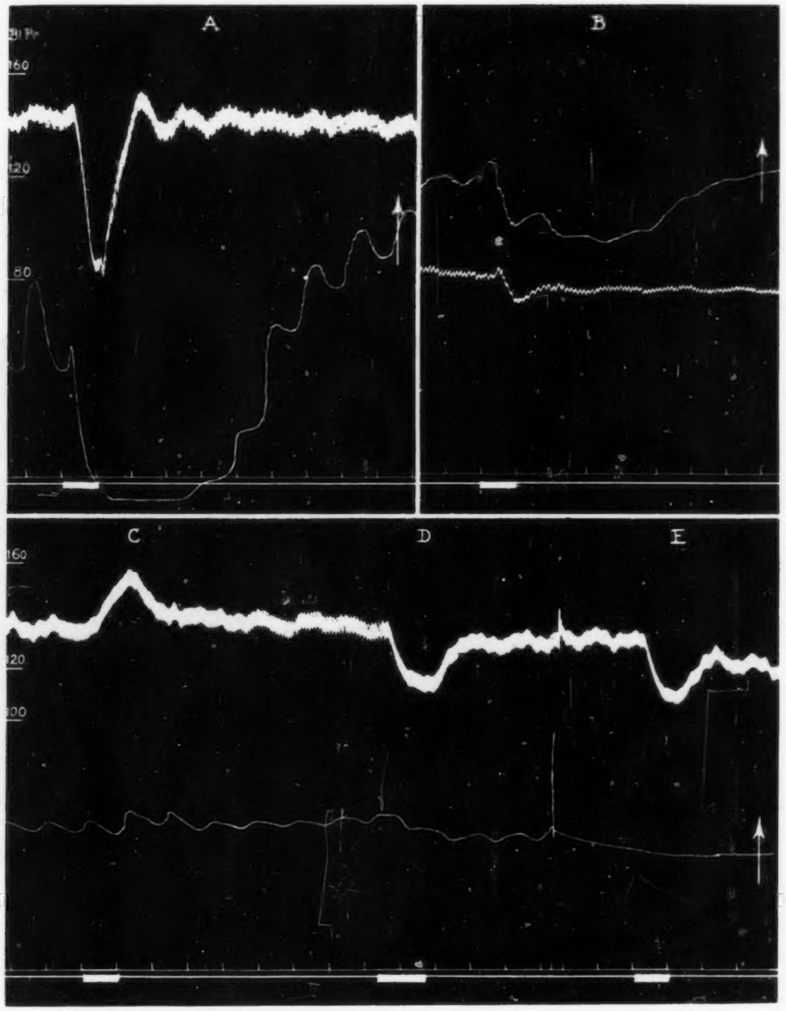

Fig. 3 A. Completely sympathectomized; dial; vagi cut. Left vagus stimulated centrally. Upper record: blood pressure. Lower record: plethysmogram of the spleen; the arrow indicates constriction.

B. Abdominal sympathetics removed and splanchnics cut previously. Dial; vagi cut; spinal cord transected at L4. Stimulus as in A. Upper record: plethysmogram of the spleen; the arrow indicates constriction. Lower record: blood pressure.

C. Same animal and records as in A. Right crural nerve stimulated centrally with a tetanizing frequency and a coil distance of 6 cm. (Harvard inductorium; 5 volts in the primary circuit).

D. Same animal and records as in A. Right crural nerve stimulated centrally with 8 induction shocks per second; coil distance 8 cm.

E. Same animal as in A. Same stimulation as in D. Upper record: blood pressure. Lower record: plethysmogram of the leg.

jected. Cats with the sympathetic system intact present a marked fall under similar circumstances (see Rosenblueth and B. Cannon, 1933).

C. *Changes in volume of the spleen and kidney* (with the assistance of M. McK. Sawyer). In vagotomized and completely sympathectomized cats reflex falls of blood pressure induced by central stimulation of the left vagus and depressor nerves are sometimes attended by dilatation of the spleen (fig. 3A; cf. Bacq, Brouha and Heymans, 1933b) and of the kidney. The same stimulation did not usually affect the volume of the hind leg or

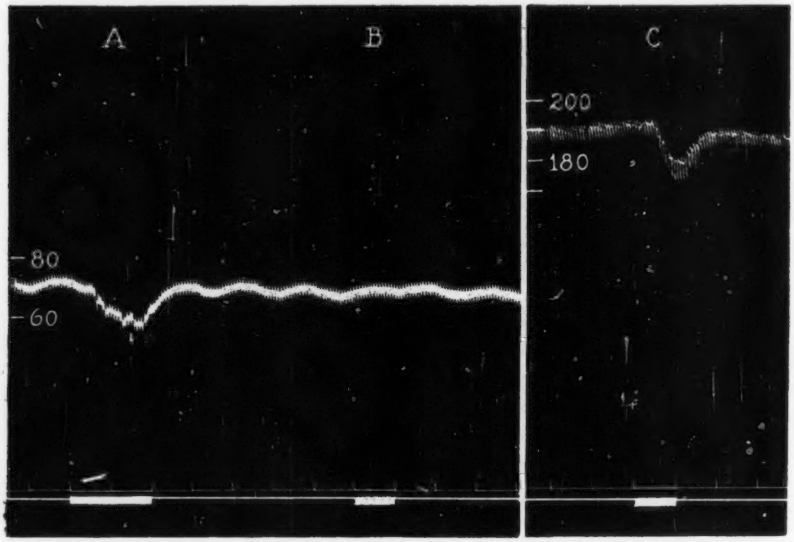

Fig. 4. Completely sympathectomized; dial; vagi cut; carotids tied; cerebellum removed, exposing the floor of the 4th ventricle; bipolar electrodes.

A. Stimulation of the depressor points. Secondary coil at 9 cm. and 60°. No movements of the animal.

B. Left pressor point stimulated with the same coil distance and angle as in A. No movements of the animal.

C. Stimulation of the depressor points after the aorta had been clamped at the diaphragm. No movements of the animal.

it evoked a slight constriction (cf. Bacq, Brouha and Heymans, 1932b). Reflex falls or rises of blood pressure caused by central stimulation of the crural nerve did not reveal any distinctive effects on either the spleen or the leg (fig. 3C, D and E). The dilatation of the spleen on central stimulation of the vagus was found to persist after section of the spinal cord at L4 (fig. 3B).

D. *Effects of stimulation of the pressor and depressor centers in the medulla.* As shown by Ranson and Billingsley (1916), there are on each side in the floor of the fourth ventricle two points, anterior and posterior, stimulation of which causes a rise and a fall of blood pressure, respectively. In our experiments on stimulating these points in sympathectomized animals, the cerebellum was removed under dial anesthesia after section of the vagi. Artificial respiration was used throughout the experiments. Both unipolar and bipolar stimulations were employed.

Electrical stimulation of the depressor points consistently elicited a fall of blood pressure (fig. 4A). Special precautions were taken to use threshold stimuli in order to avoid muscular movements due to spread of the current, since movements are usually associated with a fall of blood pressure in sympathectomized cats (Freeman and Rosenblueth, *loc. cit.*). When the electrodes are placed properly, and threshold stimuli are employed, the response is readily obtained without any visible movement. The fall persisted after occlusion of the aorta by a clamp at the level of the diaphragm (fig. 4C).

Stimulation of other neighboring regions, including the pressor points (fig. 4B), did not produce any change of blood pressure, unless the strength of the current was sufficient to induce muscular activity. In that case there appeared usually an initial rise succeeded by a fall when the stimulus ceased. A similar effect was obtained from peripheral stimulation of sectioned motor nerves, e.g., the sciatic.

Discussion. From the facts mentioned in the introduction the conclusion is reasonable that there are dilator centers connected with fibers which distribute widely in the organism and which do not belong to the sympathetic system. The results reported in section D support this conclusion. The depressor point is sharply localized and lies lateral to the apex, in the extreme posterior part of the fourth ventricle, anterior to the nuclei of Goll and Burdach—i.e., above the relay of the sensory afferent fibers from the dorsal roots. We may then infer that the fall of blood pressure obtained from stimulation of the depressor point (fig. 4) is not due to direct stimulation of dorsal root fibers. This inference is in accord with the absence of results when the electrodes are applied lower on the dorsal surface of the spinal cord (section D). Indeed, if the depressor point should not be properly a center, but a region where afferent vagal depressor fibers occupy a superficial position in the floor of the fourth ventricle, as has been suggested (Scott and Roberts, 1923; Scott, 1924), the possibility of a direct stimulation of dorsal root fibers is entirely excluded.

It is well known that a sufficient dose of curare abolishes the dilator response to dorsal root stimulation (see Bayliss, 1923). The rise of blood pressure caused by curare in sympathectomized animals (fig. 1) may therefore be explained as due to a paralysis of the dilator tone (cf. Freeman and Rosenblueth, *loc. cit.*). In normal animals, with a predominant con-

strictor tone, the slight fall of blood pressure occasioned by curare may be due to a relaxation of skeletal muscles that increases the capacity of the vascular system.

That section of the cervical spinal cord may elicit a transient fall of blood pressure in sympathectomized animals, curarized and with vagi cut (fig. 2A), is probably due to dilator stimulation when paralysis is incomplete. The cases in which no noteworthy changes of blood pressure occur are in keeping with a complete paralysis of the dilators by curare and with the lack of constrictors in such preparations.

The negative results obtained on stimulation of the spinal cord of sympathectomized animals under curare (section A) may be explained as due to the paralyzing effects of curare on the dilator responses. The one positive case was probably a consequence of an incomplete paralysis from a too small dose of curare.

The effects of ergotoxine (section B) lead to the inference that the fall of blood pressure obtained in the myelencephalic, unanesthetized preparation (Rosenblueth and B. Cannon, *loc. cit.*) is due to the influence of sympathetic dilators. This suggests the possibility of two dilator centers, one connected with sympathetic, the other with non-sympathetic fibers. Further evidence is necessary, however, before such a distinction can be established.

The only widely distributed non-sympathetic vasodilator nerve fibers known are the dilators in the dorsal roots. That these dilators may be activated reflexly has been shown by Bayliss (1902), Fofanow and Tschalussow (1913), Bishop, Heinbecker and O'Leary (1933) and Tournade and Malméjac (1933). We assume, therefore, that in sympathectomized animals these are the efferent paths of the vasomotor reflexes (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1932a), of the dilator impulses responsible for the fall of blood pressure attending struggle (*ibid.*), and of the response to stimulation of the depressor point in the floor of the fourth ventricle (fig. 4A and C).

Some alternative interpretations have been suggested. Bacq, Brouha and Heymans (1932b) report a failure to obtain reflex changes of volume in sympathectomized limbs of cats and dogs. They conclude that the sympathetic contains all the vasomotor fibers to this territory that are capable of reflex activation. Their evidence is unfortunately negative, and contradicts the positive results obtained by Bayliss (1902), Fofanow and Tschalussow (1913), Bishop, Heinbecker and O'Leary (1933) and Tournade and Malméjac (1933). In our experiments (section C) the cases in which reflex falls or rises of blood pressure did not result in any change in the volume of the leg (fig. 3E) are to be interpreted as due to corresponding dilatation and constriction, since the passive changes do not appear.

Bacq, Brouha and Heymans (1933a) suggest further that the fall of

blood pressure, which occurs when sympathectomized animals with the vagi cut struggle, may be due to a direct peripheral effect of the metabolites of muscular contraction—e.g., CO_2 and the H ion (cf. Barcroft, 1914; Gaskell, 1920; Langley, 1923). CO_2 may or may not have a direct dilating effect on the vessels. The evidence adduced by Bacq, Brouha and Heymans does not demonstrate this effect. They reproduce a record showing a fall of blood pressure and a largely increased respiratory rate when CO_2 was added to the air breathed by a sympathectomized dog. The fall of blood pressure may then have been due, as they conclude, to a direct, generalized dilating effect of the CO_2 on the arterioles; but it might also have been the consequence of the muscular activity associated with the increased respiratory movements; it might, finally, be due to a central excitation of dilators by the CO_2. The fall of blood pressure in sympathectomized, vagotomized animals when they struggle is too rapid and immediate to be easily explained by the metabolites of muscular contraction. When the peripheral end of the cut sciatic is stimulated in a sympathectomized cat, there is first a rise of blood pressure; the fall only occurs after the stimulus ceases (section D).

Bacq, Brouha and Heymans (1933b), finally, assume that the vasomotor reflexes of sympathectomized and vagotomized cats are limited to the abdominal viscera (e.g., spleen) and are mediated by fibers from the sacral parasympathetic. These suggestions seem to us quite doubtful, both anatomically and physiologically. Anatomically, nervous pathways connecting the sacral autonomic division and the spleen are unknown. Physiologically, vasodilator responses persist in sympathectomized cats with the vagi cut, after clamping of the aorta at the diaphragm has excluded the abdominal viscera from the circulation (section D, fig. 4C); furthermore, the dilatation of the spleen on central stimulation of the depressor nerve may persist after section of the spinal cord at L4 has disconnected the sacral autonomic outflow from the higher centers (fig. 3B). If this dilatation of the spleen should be of nervous origin the only nerve fibers known that might account for it are the connections which have been described between the phrenics and the semilunar ganglia (see Poirier and Charpy, 1921). It is possible, however, that the effect may be passive, due to changes in the intra-abdominal pressure or to modifications of the portal venous pressure. Further experiments are necessary to elucidate the phenomenon.

All the data available can readily be explained if we assume that the dilator fibers in the dorsal roots may be activated centrally (Bayliss, 1902). With the anatomical evidence at hand it is not possible to determine the precise nature of the fibers concerned—i.e., whether the impulses are antidromic (Bayliss, 1901), or are conveyed by possible efferent fibers in the dorsal roots (Kuré, Nitta, Tsuji, Shiraishi and Suenaga, 1928; Gagel, 1930).

We suggest that the vasodilator system is probably constituted and distributed as follows: A, *autonomic dilators,* (1) sympathetic, distributed primarily to skeletal muscle (see Cannon and Rosenblueth, 1933; Rosenblueth and B. Cannon, 1933), (2) parasympathetic, distributed to special, localized regions (e.g., chorda tympani, nervi erigentes); B, *dorsal root dilators,* distributed primarily to the skin and the viscera (Bayliss, 1901 and 1902).

SUMMARY

The following vasomotor effects were obtained in completely sympathectomized cats with the vagi cut: curare elicits a rise of blood pressure (fig. 1); section of the cervical spinal cord evokes no change or a slight transient fall (fig. 2); ergotoxine causes a considerable rise (section B); reflex falls may be attended by dilatation of the spleen (fig. 3A and B); reflex rises and falls may not be accompanied by any distinctive volume changes of either the spleen or the leg (fig. 3C, D and E); stimulation of the depressor points in the fourth ventricle causes a fall of blood pressure (fig. 4A and C), whereas stimulation of neighboring regions, including the pressor points (fig. 4B) is negative, unless muscular activity is evoked (section D).

Possible explanations for these phenomena are discussed (pp. 603–604). The conclusion is reached that, in sympathectomized animals, the dilator fibers in the dorsal roots are probably the efferent paths of the response to stimulation of the depressor points, of the vasomotor reflexes, and of the dilator impulses responsible for the fall of blood pressure attending struggle (pp. 604–605).

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A FAILURE TO CONFIRM PAVLOV'S HYPOTHESIS OF EXTERNAL INHIBITION

ABEL R. MILLER

From the Department of Physiology of the Ithaca Division of the Cornell Medical College and the Department of Physiology of Michigan State College

Received for publication September 30, 1933

The publication of I. P. Pavlov's two books concerning the conditioned reflex (1, 2) has at last made available the data upon which his theory of the physiological activity of the cerebral cortex is founded.

In 1928 the experiments described below were begun at Ithaca and have been continued in the Department of Physiology at East Lansing since the fall of 1929 with the aim of attempting to establish positive and negative conditioned salivary reflexes in the dog by the methods and with the precautions so exactly described by Pavlov and by his colleague Podkopaev and of attempting to show the relation between their constancy and the constancy of the experimental environment (4).

Since such experiments must, according to Pavlov, be conducted under constant conditions, rooms were chosen in the Ithaca and East Lansing laboratories in which the various environmental conditions, particularly the noise level, could be controlled.

External inhibition. From the first Pavlov has stressed the importance of a constant environment for the successful investigation of conditioned reflexes (3). The fluctuations in magnitude of such conditioned reflexes due to distraction (external inhibition) have recently been confirmed by Volborth (5).

In the absence of further direct experimental confirmation of these findings, one is constantly tempted to explain any change in the magnitude of the conditioned reflex as due to some subtle change in the environment to which the dog reacts but which cannot be noticed by the experimenter. And since Pavlov's theory of the conditioned reflex depends so directly upon the interpretation of changes in the magnitude of conditioned reflexes (the amount of saliva secreted), this factor of external inhibition merits the most serious investigation if one is to attempt to confirm the principal points of this complex theory.

In the experiments of Liddell and Anderson[1] it early became questionable whether or not external inhibition exists in the motor conditioned reflexes

[1] Unpublished experiments, Cornell University.

in the sheep. Anderson then attempted to demonstrate the phenomenon during the formation of conditioned motor reflexes in the dog with essentially the same results as in the sheep; namely, that there is little or no effect observed from the action of distracting agents (external inhibitors). In the present group of experiments the evidence which we shall present is equivocal and again brings the great importance attached to this phenomenon in question.

1. *Effect of external inhibition on newly formed reflexes.* In this case the reflex was first elicited at the seventh presentation of the metronome and was demonstrated to a class of students eight days later with the following results:

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX IN 0.01 CC. UNITS OF SECRETION
2:33	24M60*	30″	3″	0.36
2:38	25M60	30″	3″	0.40

* 24M60 indicates that the metronome beating 60 to the minute is presented for the 24th time.

Immediately after the last stimulation the class was allowed to observe the dog through the open door and then left the room, obviously affording a great many stimuli to which the dog is not accustomed in the laboratory environment. As a result marked external inhibition was recorded:

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX
2:43	26M60	30″	20″	0.08

2. *External inhibition produced by a new conditioned stimulus.* This example was recorded during the formation of a new positive conditioned reflex to the sound of a buzzer and had the following effect on the metronome reflex despite the fact that the sound of the metronome had been accompanied by food 357 times.

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OE THE CONDITIONED REFLEX
3:57	357M60	30″	7″	0.10
4:02	1 buzzer	5″	—	0
4:08	358M60	30″	—	0
4:15	359M60	30″	—	0
4:19	2 buzzer	5″	—	0

The sound of the buzzer was always reinforced with food but the new sound produced such a marked inhibition that the dog refused to eat. Upon subsequent repetitions of the buzzer it rapidly became a conditioned signal for food and proved to be a more potent stimulus than the metronome as shown by the following protocol:

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX
5:15	376M60	30″	3″	0.20
5:19	18 buzzer	30″	2″	0.58
5:24	377M60	30″	4″	0.22
5:30	19 buzzer	30″	3″	0.28
5:38	378M60	30″	—	0
5:42	20 buzzer	30″	12″	0.20

Note the total inhibition to the 378th presentation of the metronome at 5:38. Is this external inhibition and if so why should it occur in the absence of any sudden change in the laboratory environment?

3. Effect of unusual stimuli on a well established reflex. The effect of unusual stimuli was then tested, the door to the chamber was left open and the experimenter created noises during the interval between the applications of the conditioned stimulus, *viz.*, the metronome which had served as a signal for food on 120 previous occasions. In each case the dog turned toward the source of the noise but, as will be seen from the protocols below, the effect of these orienting reactions on the magnitude of the conditioned reflex was doubtful.

Tuesday, May 17th:

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX	REMARKS
4:42	121M120	30″	3″	0.27	Usual conditions
4:48	122M120	30″	2″	0.19	Experimenter rattled chair and whistled loudly during sounding of metronome
4:55	123M120	30″	9″	0.18	Usual conditions

Since the last reflex under the usual conditions, seven minutes after the disturbing noise, was reduced to 0.18 cc. as compared with the first reflex of the day, 0.27 cc., its reduction might be due to the after effects of the whistling and rattling or it might be a fluctuation in magnitude due to unknown causes. It is interesting to observe that the reflex coinciding

with the whistling and rattling is 0.19 cc. as compared with the initial "normal" reflex of 0.27, whereas seven minutes after the distraction has ceased the reflex is still reduced (0.18 cc.).

Monday, May 23rd:

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX	REMARKS
4:15	141M120	30″	4″	0.35	Usual conditions
4:22	142M120	30″	6″	0.14	Experimenter scratched on outside of room
4:27	143M120	30″	6″	0.22	Experimenter whistled loudly

Finally heroic measures were adopted in attempting to demonstrate the external inhibition of a well established conditioned reflex. A tambour device was cemented to the skin of the dog at the lower border of the ribs by which tactile stimuli could be administered when the experimenter pressed a bulb in the adjoining room. A large pendulum suspended over the food dish could be started to swinging; thus providing visual stimuli. An electromagnetic tapper knocked on the under side of the table and a box of peas under the table was shaken by a pulley arrangement. The protocol given below shows the small effect on the magnitude of the conditioned reflex produced by this massive distraction.

TIME OF STIMULATION	CONDITIONED STIMULUS	DURATION OF CONDITIONED STIMULUS	LATENT PERIOD	SIZE OF THE CONDITIONED REFLEX IN 1/70TH CC. DIVISIONS
10:07	182M120	30″	9″	15
10:16	183M120	30″	10″	10
10:22	184M120	30″	9″	9

Note: At 10:07 and 10:22 usual conditions prevailed. For a period of 15 seconds preceding the application of the conditioned stimulus at 10:16 the dog was subjected to novel auditory, visual, and cutaneous stimuli presented in irregular sequence which continued until about one minute before the presentation of 184M120. Between 183M120 and 184M120 the dog came to the edge of the table to investigate the noises under the table. The tactile stimulus was then presented and the dog, startled, fell off the table and hung by his collar. The experimenter entered the room, put him back on the table and about one minute later stimulus 184M120 was presented under normal conditions; *i.e.*, without distractions.

CONCLUSION

The aim of this investigation was to reproduce as faithfully as possible Pavlov's technique for the establishment of conditioned salivary reflexes.

In general, the findings of Pavlov and his colleagues have been verified with one important exception. One of the building stones of his complex structure of theory to account for cerebral activity is the phenomenon of external inhibition. With every expectation of easily verifying this phenomenon we have signally failed to demonstrate its uniform occurrence. In making this statement the intention is not to question the trustworthiness of Pavlov's results but to emphasize again the importance of repeated verification of experiments in this most complex field of physiological endeavor as a means of disclosing unsuspected factors responsible for variable results.

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THE EFFECT OF THYROXINE INGESTION ON THE TOXICITY OF CERTAIN BILE SALTS

L. H. SCHMIDT

From the Christ Hospital Research Institute, Cincinnati, Ohio and the Department of Biochemistry, University of Cincinnati

Received for publication March 3, 1934

In preliminary experiments, Tashiro and Schmidt (1) established a relationship between thyroid gland activity and bile salts toxicity. Using gastric ulcer formation as the criterion of toxicity, they demonstrated that ingestion of thyroxine by a male guinea pig increased the toxicity of sodium glycocholate and sodium taurocholate, injected intraperitoneally. This observation was of interest for two reasons. In the first place, there is a variety of pathological conditions in which bile salts may accumulate in the blood stream. In these cases it is necessary to recognize any condition that will alter bile salts toxicity. Secondly, this observation suggests that thyroxine may represent a physiological agent which is concerned with the normal metabolism of the bile salts. Therefore, it would be of considerable interest to determine the manner in which thyroxine affects bile salts toxicity.

With this in view, the present investigation has had the following purposes: first, to study in a more quantitative manner the relation of thyroxine ingestion to sodium glycocholate and sodium taurocholate toxicity; second, to compare effects of thyroxine ingestion on this toxicity in male and female animals; third, to determine whether thyroxine ingestion increases toxicity of other bile salts than the above; fourth, to study the quantitative relation between the amount of thyroxine ingested and the amount of phosphatide antagonizer required to detoxify a given amount of bile salts.

EXPERIMENTAL: MATERIALS AND METHODS. *Thyroxine.* Oral thyroxin, Squibb, was used in all experiments. The desired amounts were suspended in tap water and fed to each animal with a medicine dropper.

The loss of body weight of the guinea pigs, following thyroxine ingestion, was noted and served as an index of the effectiveness of treatment. The maximum loss of weight usually occurred 72 hours after the last thyroxine ingestion.

Bile salts. The sodium salts of taurocholic and glycocholic, cholic, deoxycholic and dehydrocholic acids were used in this study. Sodium taurocholate and sodium glycocholate (Fairchild Bros. & Foster) were used

in a mixture containing approximately 2 parts of the taurocholate to 3 parts of the glycocholate. Cholic acid (M. P. 184–187°C.) and deoxycholic acid (M. P. 169–170°C.) were prepared from ox bile by the method of White (3). The sodium salts were formed at the time of preparation of the solution for injection. A slight excess of 0.1 N. sodium hydroxide was added to the required amount of cholic or deoxycholic acid, then neutralized to phenolphthalein with 0.1 N. hydrochloric acid. These solutions were sterilized and then made up to a definite volume with 0.9 per cent sodium chloride. The sodium dehydrocholate used was a commercial preparation (*Decholin*—Riedel-de Haen). All of these bile salts solutions were injected intraperitoneally under aseptic conditions.

Lecithin. Brain lecithin was used as an antagonizer of bile salts toxicity. It was prepared by the method of Levene and Rolf (4) with incorporation of the modifications suggested by Maltaner (5). This preparation contained 1.71 per cent nitrogen and 3.92 per cent phosphorus. Traces of cephalin were probably present, since 0.14 per cent of the nitrogen occurred as amino nitrogen.

When lecithin was used, the desired quantity of a freshly prepared 2 per cent alcoholic solution was placed in a small beaker—either alone or with a definite quantity of bile salt. The alcohol was removed on a steam bath and the residue emulsified in 0.9 per cent sodium chloride solution, the emulsion transferred to a graduated test tube, sterilized, and made up to volume with sterile saline.

Experimental animals. Both male and female guinea pigs were used in this study.

Preliminary experiments demonstrated that the toxicity of bile salts administered alone and after thyroxine ingestion varied with age groups. Results obtained with young pigs weighing between 175 and 275 grams were inconsistent. Results obtained with animals weighing 500 to 700 grams were reliable only when the animals were of approximately the same age and had been reared under similar conditions. Therefore, the present experiments were performed only on animals weighing between 325 and 450 grams.

Experiments were carried out from December to April inclusive. During this period the temperature of the animal quarters was maintained at 22°C. ± 2.5°. The factor of seasonal variation and especially temperature control is very important for comparison of effects of thyroxine ingestion on bile salts toxicity. As will be pointed out in a later paper, the effects of thyroxine ingestion on the guinea pig are more marked in the summer months.

The diet of the guinea pigs consisted of an unlimited supply of alfalfa hay and prepared rabbit food (Pratt's Rabbit Pellets), supplemented by daily rations of approximately 10 grams lettuce per pig.

Criterion of toxicity. Formation of gastric ulcer, following injection of bile salts, served as the criterion of toxicity. (Sellards (6) had first observed that this action could be produced by injection of bile or crude bile salts.) Ulcer formation is a more reliable and sensitive measure of toxicity than death. As Tsuruta (2) previously observed, the amount of bile salt required to kill normal male guinea pigs varies between 10 and 22 mgm. per 100 grams body weight, but the amount of the same bile salt required to produce gastric ulcers is nearly always 17.5 to 19 mgm. Using the criterion of gastric ulcer formation, it is necessary to kill every surviving animal injected with bile salts. Control animals receiving thyroxine only, and normal controls were frequently killed, but under the conditions of these experiments, no ulcers were found in these groups.

Death resulting from bile salt injection invariably occurs within 48 hours of the time of injection. Gastric ulceration occurs as early as 5 hours after bile salts injection. However, these ulcers heal rapidly; 144 hours after injection healing processes are so well advanced that macroscopic identification of the ulcer is difficult. Therefore, all surviving animals were chloroformed 60 hours after bile salt injection. The stomachs were quickly removed and examined macroscopically. Only actual breaks in the stomach mucosa were termed ulcers. Whenever doubt existed as to the presence of a mucosal break, the tissue was fixed in 10 per cent formalin and microscopic sections were made. These lesions usually appeared on the greater curvature of the stomach in the lower portion of the fundus region, almost opposite the pyloric sphincter. The microscopic character of these lesions will be described in detail elsewhere.

Procedure for determining the amount of bile salt required to produce a gastric ulcer. Since the maximum effects of thyroxine ingestion on bile salts toxicity are to be observed 48 to 60 hours following the last thyroxine feeding, all bile salts injections were made 48 to 54 hours after this last feeding. The amount of bile salt injected was based on the body weight of the animal at the time of injection.

Preliminary experiments determined approximately the amount of a bile salt required to produce gastric ulcers in a certain group of animals; i.e., thyroxine or normal control group. The exact amount of bile salt required was then determined in a larger group of animals. This latter result was always confirmed by subsequent experiments.

A similar procedure was followed in determining the minimum amount of lecithin that would inhibit the action of bile salts in producing gastric ulcers.

The following data are summaries of results obtained with five hundred eighty male and female guinea pigs.

RESULTS. *A. The effect of thyroxine ingestion on sodium glycocholate and sodium taurocholate toxicity.* Ingestion of thyroxine by either male or

female guinea pigs increases the toxicity of mixtures of sodium glycocholate and taurocholate. This increase in toxicity is roughly proportional to the amount of thyroxine ingested. However, as larger amounts of thyroxine are administered, changes in bile salt toxicity become smaller. These results have been summarized in figure 1.

a. Male. Production of a gastric ulcer in a normal male guinea pig requires the injection of 17.5 mgm. of sodium glycocholate and taurocholate per 100 grams body weight. If 0.2 mgm. thyroxine has been ingested 48 hours prior to the bile salts injection, 13.5 mgm. of the bile salts mixture are sufficient to produce the ulcers. After ingestion of 0.4 mgm. of thyroxine

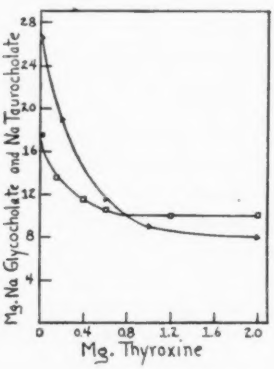

Fig. 1

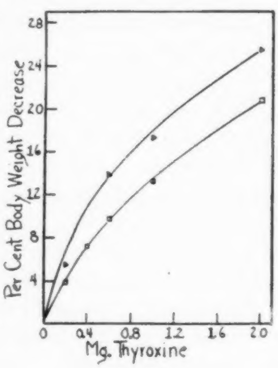

Fig. 2

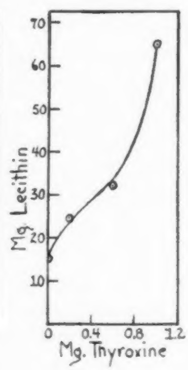

Fig. 3

Fig. 1. The effect of ingestion of thyroxine on the minimum amount of sodium glycocholate and sodium taurocholate required to produce gastric ulcers in the guinea pig. △ = female, □ = male.

Fig. 2. The effects of thyroxine ingestion on the body weight of the guinea pig. △ = female, □ = male.

Fig. 3. The effects of thyroxine ingestion on the amount of lecithin required to detoxify injections of 35 mgm. of sodium glycocholate and sodium taurocholate.

ine (at the rate of 0.2 mgm. daily on each of 2 successive days) only 11.5 mgm. of the bile salts are required to produce ulceration. Ingestion of 0.6 mgm. of thyroxine in a period of 3 days increases toxicity so that 10.5 mgm. of bile salts produce gastric ulcers. However the ingestion of 1.0 or 2.0 mgm. of thyroxine (0.2 and 0.4 mgm. on each of 5 successive days) does not provoke proportional increases in bile salts toxicity. In no instance have gastric ulcers been formed in male animals with less than 9 mgm. of bile salts per 100 grams body weight.

This last observation is difficult to explain. It may be suggested that maximum physiological changes in the guinea pig are stimulated by ingestion of slightly less than 1.0 mgm. thyroxine. However, the decrease

in body weight following ingestion of varying quantities of thyroxine indicates that such is not the case (fig. 2), since ingestion of 0.4, 1.0 and 2.0 mgm. of thyroxine decreases the body weight by 7.2, 13.2 and 20.9 per cent respectively.

b. Female. Tsuruta (7) observed that the amount of sodium glycocholate required for gastric ulcer formation is larger for the female guinea pig than for the male. The present observations show that injection of 26.5 mgm. of the mixed bile salts per 100 grams body weight is necessary to produce gastric lesions in the normal female guinea pig, whereas only 17.5 mgm. are required for the normal male.

After a single ingestion of 0.2 mgm. of thyroxine, 19 mgm. of bile salts per 100 grams body weight will produce gastric ulcer (fig. 1). Three ingestions of 0.2 mgm. of thyroxine on successive days reduce the amount of bile salt required to 11.5 mgm. Injection of 9.0 mgm. of the bile salts mixture produces ulceration after the female has ingested 1.0 mgm. of thyroxine. Thus *the effects of thyroxine ingestion on bile salt toxicity are more marked in the female than in the male guinea pig.*

The average loss in body weight following thyroxine ingestion is also greater in the female pig than in the male. Ingestion of 0.6, 1.0 and 2.0 mgm. of thyroxine by a female animal decreases the body weight 13.7, 17.2 and 25.1 per cent respectively (fig. 2). In the male, similar doses of thyroxine decrease the average body weight 9.7, 13.2 and 20.9 per cent.

B. The effect of thyroxine ingestion on sodium cholate, sodium deoxycholate and sodium dehydrocholate toxicity. Experiments were carried out to determine whether the above action of thyroxine is peculiar to the salts of conjugated cholic acids alone, or whether it is also a property of unconjugated bile salts.

Tsuruta (8) demonstrated that sodium cholate and sodium deoxycholate produce gastric ulcers when injected intraperitoneally. In the present experiments, injection of 11.0 mgm. of sodium cholate per 100 grams body weight is necessary to produce ulceration in normal male guinea pigs. However, after ingestion of 1.0 mgm. of thyroxine, injection of only 6.0 mgm. of sodium cholate produced ulcers. Similarly, in experiments with sodium deoxycholate, 6 mgm. were required to produce gastric lesions in the normal male guinea pig, but only 3 mgm. were required after ingestion of 1.0 mgm. of thyroxine.

The above observations, summarized in table 1, are in marked contrast to the results obtained with sodium dehydrocholate. This salt of the triketocholanic acid has a very low toxicity. Injection of 100 mgm. per 100 grams body weight is required to produce gastric ulcer in the normal male animal and the animal that has ingested 1.0 mgm. of thyroxine. Thus, *the toxicity of sodium dehydrocholate is not altered by thyroxine ingestion.*

C. Effects of thyroxine ingestion on the capacity of lecithin to inhibit bile

salt toxicity. In their preliminary report, Tashiro and Schmidt (1) suggested that thyroxine ingestion increased bile salt toxicity by disturbing the lipid metabolism in such a manner that the amount of phospholipid in blood and tissues is decreased. This viewpoint was supported by two lines of evidence. First, the phospholipids, lecithin and cephalin, the crude sulfolipids (Tsuruta, 2) and the cholesterol esters, (Ishii, 9) are the only substances thus far studied that will inhibit gastric ulcer formation following bile salts injection. Secondly, the experiments of Bing and Heckscher (10, 11), Tashiro and Schmidt (12) and Sakurai (13) indicate that thyroxine ingestion or injection produces decreases in the cholesterol and phospholipid content of whole blood.

If the suggestion of Tashiro and Schmidt were correct, it should follow that the amount of lecithin or other phospholipid required to detoxify a given quantity of bile salt would increase following ingestion of varying quantities of thyroxine. Experiments with male guinea pigs demonstrate

TABLE 1

The effects of thyroxine ingestion on the minimum amounts of bile salts required to produce gastric ulcer

BILE SALT USED	NORMAL MALE GUINEA PIG	MALE GUINEA PIG AFTER INGESTION OF 1.0 MGM. THYROXINE
	mgm.	*mgm.*
Sodium cholate	11.0	6.0
Sodium deoxycholate	6.0	3.0
Sodium dehydrocholate	100.0	100.0
Sodium glycocholate and taurocholate	17.5	9.0

that such is the case (fig. 3). If 35 mgm. of the sodium glycocholate and taurocholate mixture are injected into a normal male guinea pig, a simultaneous injection of 15.5 mgm. of lecithin per 100 grams body weight inhibits gastric ulcer formation. However, after ingestion of only 0.2 mgm. of thyroxine, 24 mgm. of lecithin are required to detoxify each 35 mgm. of bile salt. After ingestion of 0.6 mgm. of thyroxine, 32 mgm. of lecithin are required. Ingestion of 1.0 mgm. of thyroxine necessitates using 65 mgm. of lecithin with each 35 mgm. of bile salt.

However, a second observation indicates that disturbances in lipid metabolism are not solely responsible for the increase in bile salts toxicity produced by thyroxine ingestion. *Lecithin inhibits the formation of gastric ulcers following sodium dehydrocholate injections.* Simultaneous injection of 10 mgm. of lecithin with 150 mgm. of sodium dehydrocholate per 100 grams body weight prevents gastric ulcer formation. Nevertheless, *ingestion of thyroxine did not increase sodium dehydrocholate toxicity.* It

should be increased if the increase in other bile salts toxicity is primarily due to decreases in blood and tissue lipids.

Discussion. These experiments have demonstrated four things: 1, that thyroxine ingestion increases bile salts toxicity in both sexes; 2, that quantitatively the female animal reacts to thyroxine more severely than the male; 3, that the effect of thyroxine on the bile salts depends to a certain degree on their molecular structure; 4, that the amount of lipid (lecithin) required to detoxify a given quantity of bile salts increases following thyroxine ingestion. These facts have not entirely explained the manner in which thyroxine increases bile salts toxicity.

When Tsuruta observed that normal female guinea pigs and frogs were more resistant to bile salts actions than male animals, he suggested that this sex difference was due to different blood and tissue lipid contents. Were this correct, the present experiments might indicate that thyroxine ingestion produces the greater effect on the lipid metabolism of the female. Experiments are now in progress to test this indication.

The fact that ingestion of thyroxine increases the amount of lecithin required to detoxify a given quantity of bile salts merely indicates that thyroxine has made the animal more susceptible to the bile salts. It is evident that this would be the case whether thyroxine ingestion increases bile salts toxicity by decreasing the blood and tissue phospholipids or by any other mechanism.

Although simultaneous injection of lecithin inhibited sodium dehydrocholate toxicity, thyroxine ingestion did not affect this toxicity. This fact indicates that disturbances of lipid metabolism are not solely responsible for the effect of thyroxine ingestion on bile salts toxicity.

One may suggest that the toxicity of the bile salts is determined by the rate at which they are broken up or elaborated into other products; normally, intermediary products may arise that are more toxic than the original bile salts. Perhaps thyroxine ingestion accelerates the formation of these products or prevents their alteration to less toxic forms. This process might involve splitting of the bile salts molecule at the secondary alcohol groups, and would explain the failure of thyroxine to affect sodium dehydrocholate toxicity.

Recent experiments in this laboratory have indicated another factor possibly involved in the effect of thyroxine on bile salts toxicity. The addition of small amounts of oleic and linoleic acids to sodium glycocholate and taurocholate causes a three-fold increase in toxicity and also in the amount of lecithin required for detoxification. Such a change would occur if thyroxine ingestion gives rise to an increased concentration of these fatty acids in the blood and tissues.

Greene and Snell (14) demonstrated that bile salts injected intravenously into normal animals are rapidly excreted in the bile. Other investigators

(15, 16, 17, 18) have observed that administration of thyroid gland to rabbits, rats and guinea pigs leads to degenerative changes within the liver accompanied by functional disturbances, which may involve bile salt formation and secretion. It is possible that thyroxine ingestion also affects the power of the liver to secrete bile salts added to the blood.

It is evident that further experiments are necessary to determine the manner in which thyroxine affects bile salts toxicity.

SUMMARY

1. Ingestion of thyroxine by male and female guinea pigs increases the toxicity of intraperitoneal injections of sodium glycocholate and sodium taurocholate.

2. This change in toxicity is roughly proportional to the amount of thyroxine ingested. However, ingestion of thyroxine by a female animal increases the toxicity three-fold, while similar ingestion by a male pig increases toxicity only two-fold.

3. This effect of thyroxine on bile salts toxicity is a property of both conjugated and unconjugated cholic acid salts and sodium deoxycholate.

4. Ingestion of thyroxine increases the amount of lecithin required to inhibit gastric ulcer formation following injection of bile salts.

5. Although lecithin detoxifies sodium dehydrocholate, ingestion of thyroxine does not increase this toxicity. This indicates that lipid changes alone will not explain the effect of thyroxine on bile salts action.

6. Suggestions are made as to the manner in which thyroxine may increase bile salts toxicity.

I am indebted to Dr. S. Tashiro for a critical survey of this manuscript.

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THE EFFECT OF DIGESTION ON THE BLOOD FLOW IN CERTAIN BLOOD VESSELS OF THE DOG

J. F. HERRICK, HIRAM E. ESSEX, FRANK C. MANN AND EDWARD J. BALDES

From the Divisions of Experimental Medicine, Physics, and Biophysics, The Mayo Clinic, Rochester, Minnesota

Received for publication March 26, 1934

It has long been thought that, during the digestion of food, blood is diverted from other tissues of the body to the viscera. The fact that the viscera appear congested has been taken as evidence of an increased blood supply to these organs. From a teleological point of view more blood should flow to those tissues the metabolism or activity of which is greatest, and consequently the more quiescent tissues might be expected to receive less blood in a given unit of time. The drowsiness that frequently follows a heavy meal has been attributed to a decreased supply of blood to the brain. The belief that an increased flow of blood to the viscera during digestion is produced at the expense of the blood supplying the somatic tissues has been so general as to give it the force of demonstrated fact. Up to the present time it has not been feasible actually to observe what happens to the flow of blood to a given organ following ingestion of food and during the digestive cycle. Consequently, the teachings of the past have been, of necessity, based on inferences drawn from certain observations.

Recent refinements in methods have made possible accumulation of definite experimental evidence on this problem. By utilizing the thermostromuhr method of Rein, with certain modifications, observations have been made under more nearly normal physiologic conditions than have formerly been possible with other methods commonly employed in the measurement of blood flow because this method permits observations on the trained, quietly resting, normal animal. In the present paper we are reporting observations on the blood flow in the femoral, carotid, and mesenteric arteries, and the external jugular vein of previously fasted dogs before, during, and following the ingestion and digestion of various kinds of food.

The dogs used in these experiments were trained to lie quietly for several hours. They were fasted for about eighteen hours prior to the experiment. The diathermy-thermo-element was placed on the femoral and carotid arteries, and on the jugular vein, with the animals under local anesthesia which was maintained throughout the period of observation. Because of

its prolonged effect, pantocain was used routinely as the anesthetic agent in these experiments. The unit was placed on the mesenteric artery with the animal under ether anesthesia, and the usual sterile technic was employed. Observations on the effect of food on blood flow were begun from twenty-four to forty-eight hours following operation.

RESULTS. *Femoral artery.* Control observations on the blood flow in the femoral artery, made over a period of time equal to that necessary for the feeding experiments, revealed very slight deviation from the initial level. That is, the blood flow through the femoral artery of a quietly resting, fasting dog, under standard environmental conditions, is approximately constant, varying but little over a period of four or five hours. In previous experiments on blood flow this likewise had been found to be true. In contrast to these observations, the quietly resting animal maintained under comparable conditions before and after feeding gives evidence of marked increase in the blood flow of the femoral artery following voluntary ingestion of food, provided the food is digested. Increases did not occur, however, in certain instances. When an emetic was given or the animal vomited voluntarily, it was seen that the meal had remained in the stomach undigested.

The time at which the increase in blood flow begins following the meal is influenced decidedly by the type of food ingested. In a series of experiments, a meal consisting of 500 cc. of milk, four eggs, and 5 grams of glucose was fed. Increase in blood flow of the femoral artery began usually within ten to fifteen minutes following ingestion of food and continued for a period ranging from one and a half to two and a half hours, after which there was a gradual return to about the fasting level, which was reached during the sixth hour after ingestion of the meal. The blood flow had not always decreased to the initial flow when the experiment was terminated. Typically, the highest value is nearly twice that of the control. In three typical experiments the average control value was 147 cc. per minute whereas the average high value was 281 cc. per minute, which occurred during the third hour after the food was taken. The average value during the sixth hour following the meal was 178 cc. per minute.

A similar effect on blood flow in the femoral artery followed ingestion of a meal consisting of beef extract (20 gm.), peptone (10 gm.), finely ground, raw horse meat (30 gm.), and milk (300 cc.). The chief difference in the effect of this meal and of the milk-egg-glucose meal lies in the duration of the increase in blood flow. In a series of observations after the milk-egg-glucose meal the increase in blood flow lasted from four to six hours, whereas the effect of the other meal had practically disappeared two to four hours after it was ingested. The percentage increase, however, was relatively the same in each case.

A meal consisting of 50 grams of glucose in 100 to 200 cc. of water caused

a rapid rise in the blood flow within a few minutes after the food was given. In these experiments there was a marked increase in blood flow within ten minutes after the glucose was taken. The duration of the effect varied

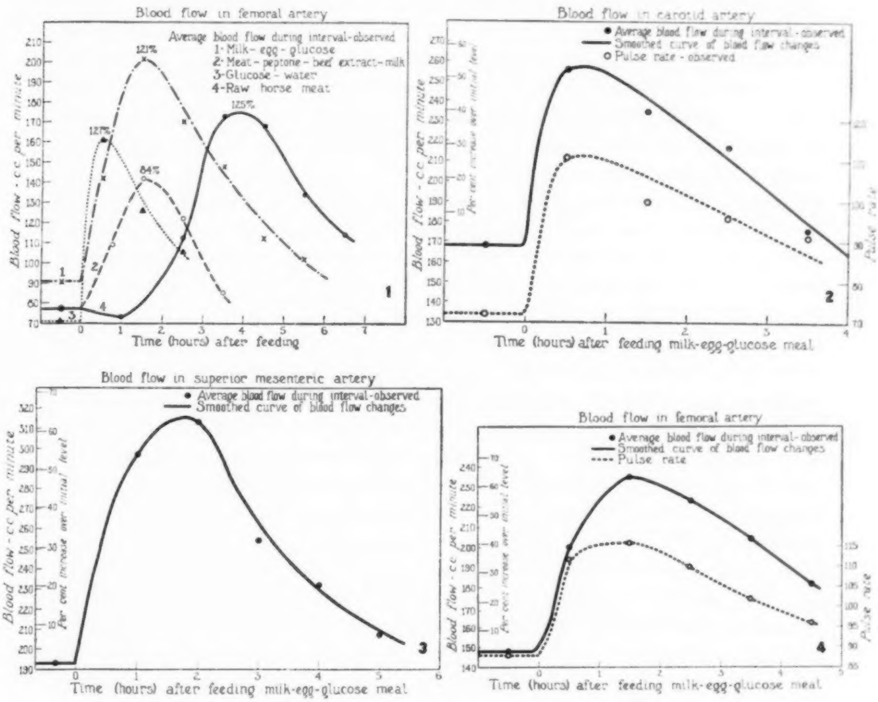

Fig. 1. The influence of the digestion of various meals on the blood flow in the femoral artery of the dog: *1*, 500 cc. milk, 4 eggs, and 5 grams glucose; *2*, 20 grams beef extract, 10 grams peptone, 30 grams finely ground, raw horse meat and 300 cc. milk; *3*, 50 grams glucose in 200 cc. water; *4*, 500 grams lean, raw horse meat without water.

Fig. 2. The effect of a meal consisting of 500 cc. of milk, 4 eggs, and 50 grams glucose on the blood flow in the carotid artery and the pulse rate.

Fig. 3. The blood flow in the mesenteric artery during the digestion of 190 cc. of a milk-egg-glucose mixture and 100 grams of a commercial dog food consisting of meat and cereals.

Fig. 4. The blood flow in the femoral artery (average values of two experiments) and the pulse rate following ingestion of a meal consisting of 500 cc. milk, 4 eggs, and 5 grams of glucose. Lumbar sympathectomy had been performed on both animals previous to the experiment.

from two to four hours. In other respects the results following this meal were similar to those obtained with the other meals just described.

In another series of experiments the blood flow in the femoral artery was

observed following ingestion of a meal consisting of 500 grams of finely ground, lean, raw horse meat. The results with this meal differed from those just described in that there was a slight initial decrease in blood flow and a much longer time was required for an increased blood flow to appear. In one case, three hours elapsed after the meal was taken before an increase in blood flow occurred. In another instance the increase occurred seventy minutes after ingestion of the meal. After the blood flow began to rise, the magnitude and duration of the increase were not significantly different from what was observed following a meal consisting of milk, eggs, and glucose (fig. 1).

Recently, Gregersen reported that a temporary drop in plasma volume of dogs occurs when no water is given during the meal or afterward. In view of our findings that an hour or more elapsed before an increase in blood flow occurred following ingestion of a meal of lean, raw horse meat, we were interested in determining whether the addition of water to such a meal would influence the onset of the increase in blood flow. Therefore the blood flow was measured in the femoral artery of two dogs before and after the ingestion of 500 grams of lean, raw horse meat to which had been added 200 cc. of water. The increase in blood flow began within fifteen minutes after the meal was taken. The maximal increase in the two experiments was 53 and 84 per cent, respectively. The blood flow had not yet returned to the original level six and five hours, respectively, after ingestion of the meal.

Common carotid artery and external jugular vein. Since the brain is partially supplied by the common carotid artery we have studied the influence of digestion on blood flow in this vessel. As in the femoral artery, blood flow in the common carotid artery is greatly augmented by digestion of the standard milk-egg-glucose meal. The magnitude and duration of the increase were comparable to those found in the femoral artery following intake of the same type of food. The control blood flow in two experiments was 90 cc. and 168 cc. per minute, whereas the maximal flow attained was 190 cc. and 256 cc., respectively. Additional experiments, in which the blood flow to the brain is studied more specifically, will be necessary before a final statement can be made concerning the influence of digestion on the blood flow to that organ. Comparable results were obtained from a study of the influence of digestion on the external jugular vein (fig. 2).

Superior mesenteric artery. We next observed the effect of digestion on the blood flow in the superior mesenteric artery, which is the main source of blood supplying the digestive tract. On the basis of previous conceptions we should expect digestion to increase the blood flow in this vessel. In three successive experiments this was found to be the case. The increase was comparable to that in the other vessels studied. Following a meal consisting of 190 cc. of a milk-egg-glucose mixture and 100 grams of a

commercial dog food composed of cooked meat and cereals, the blood flow was increased from 194 cc. to 312 cc. per minute. The increase began within ten minutes after ingestion of the food. The maximal increase was attained in ninety minutes and the blood flow had returned to nearly the original level four and a half hours after the food had been taken (fig. 3).

From the results of these experiments it is evident that the increased blood flow to the intestinal tract during digestion is not obtained at the expense of the blood flow to the somatic tissues. Since the flow in the femoral and carotid arteries and the external jugular vein is increased in a comparable manner, the evidence points clearly to a physiologic mechanism which, during digestion, produces an increase in blood flow to both somatic and visceral tissues. We have some evidence that the nutritional state of the animal influences the character of the blood flow during digestion, but these data, if found to be significant, will be reported in a later communication.

WHAT CAUSES THE INCREASED BLOOD FLOW? Since it has been proved that digestion increases the blood flow in all the vessels studied we are next concerned with the probable explanation of this phenomenon. In these experiments increases in the pulse and respiratory rates accompanied the increase in blood flow. The effect of food on the pulse and respiratory rates has been observed by various workers in connection with other studies. According to the work of Grollman, there is also a significant increase in cardiac output during digestion in man. The systolic blood pressure is slightly elevated and diastolic pressure is slightly lowered, thus producing a greater pulse pressure. An increase in cardiac output might be inferred during digestion in the dog. It seemed reasonable that the increased pulse rate might be due to peripheral vasodilatation. A plethysmographic study was made on the hind leg of a well-trained dog and on the arms of three human subjects. We did not obtain any evidence that the volume of the dog's hind leg or of the arm of a human subject was altered in the slightest by digestion of food. To obtain further evidence on this point, readings of surface temperature were made on dogs simultaneously with measurements of blood flow, but significant changes were not observed. These observations were made by placing thermocouples between the toes and on the dorsal, shaved surface of the rear feet and on a shaved area of the thorax, just above the mammary ridge.

Finally, to test whether the increased blood flow in the femoral artery following feeding might be due to a reflex vasomotor mechanism which was not detected by the other methods employed, observations were made on two dogs on which lumbar sympathectomy had been performed on the side to be studied. In such a preparation, reflex vasodilatation could not reasonably be expected to occur and, therefore, an increase in blood flow should not appear if it were due primarily to peripheral vasodilatation.

Two such experiments were sufficient to indicate that the blood flow is augmented in a manner comparable to that in the intact limb following a similar meal of milk, eggs, and glucose. The average values in two experiments show an increase from a control value of 148 cc. per minute within the first fifteen minutes to 200 cc., which gradually increased within the next hour to 235 cc. per minute, from which point there was a gradual return to 180 cc. within the next three hours (fig. 4). The combined evidence from the experiments just described is sufficient, we feel, to eliminate a reflex vasomotor mechanism as the cause of the increased blood flow in the femoral artery.

It seemed reasonable that the increased blood flow following taking of food might be due to changes in blood volume. In a series of experiments the control blood volume was determined by the dye method. From twenty-four to forty-eight hours later the blood volume was again determined from one to two hours following a milk, egg, glucose meal. A significant change was not observed in these experiments. However, we do not feel that a final statement can be made on this point because of the difficulty and the inadequacy of results obtained by this method, particularly in this type of experiment.

Considering the evidence for the time being as satisfactory, we are left with still another possible explanation of the increased blood flow, during digestion, to all the organs studied, and that is circulation time. If vasodilatation does not occur and if the blood volume does not increase, then it is conceivable that the increase in blood flow is due to a shorter circulation time. Thus, if the state of the vessels and blood volume remains the same and there is a more rapid circulation of the blood through a given organ, more blood per given unit of time should flow through that organ. The effect of digestion on the circulation time of the blood has recently been reported by Sheard, McCracken and Essex who found that the circulation time of the blood in dogs previously fasted is 20 to 30 per cent shorter than it is under fasting conditions.

Even though the increased blood flow may be accounted for on the basis of increased pulse rate, increased cardiac output, and shorter circulation time, there still remains the fundamentally important question of the factors responsible for initiating the whole process. For a time the possibility that the increase in blood flow was due to mechanical causes was considered. A distended stomach would increase the intra-abdominal pressure and might reflexly increase the cardiac rate. This theory was found untenable since, in several experiments, we did not obtain an increase in blood flow in the femoral artery following ingestion of one of the usual meals. At the end of four or five hours the effect of an emetic, or spontaneous vomiting, revealed that there had been complete retention. It is therefore evident that the increase in cardiac rate which accompanies the

increase in blood flow is not due to mechanical causes. The next possibility to be considered is the specific dynamic action of the foods ingested. If specific dynamic action were the cause of increased blood flow, it is singular that effects of the same magnitude were obtained with both carbohydrate and protein meals. Further work must be done before a definite answer can be given on this point. From the evidence thus far it would appear possible that the increase in blood flow during digestion is due to any one of the following: specific dynamic action, some product of digestion, such as a secretogogue, or, possibly, a hormone elaborated by the digestive tract.

SUMMARY AND CONCLUSIONS

A series of observations has been made on the influence of the digestion of food on the blood flow in the femoral, carotid, and mesenteric arteries and the external jugular vein. Contrary to current belief we have found that the blood is not diverted from the somatic tissue to the visceral organs during the digestion of food. Instead of a decrease in blood flow in the femoral and carotid arteries and the jugular vein there is a marked, prolonged increase during the digestive cycle. This is likewise true in the mesenteric artery. The time of the onset and the duration of the increased flow is influenced by the character of the food taken, but the magnitude is relatively the same regardless of the type of food ingested. In general the highest values for blood flow obtained during digestion are about twice those obtained after an eighteen hour fast. The initial increase in blood flow following a semifluid meal rich in carbohydrate occurs more rapidly (ten to twenty minutes) than that following a lean meat meal which may require as much as three hours. The addition of water to the meat meal causes an increase in blood flow ten to twenty minutes after the food is ingested. The duration of the increase varies from two to six hours. The increased blood flow is accompanied by an increase in pulse and respiratory rates. The rise in blood flow does not appear to be due to a reflex vasomotor mechanism, since changes in volume of limbs in man and the dog were not observed following the ingestion of food. Significant changes in surface temperature were not found. The effect of food on the blood flow in the femoral artery of dogs on which lumbar sympathectomy had previously been performed was comparable to that observed in the intact animal after ingestion of food. Significant changes in the blood volume were not apparent by use of the dye method. The increase in blood flow following a meal is accompanied by an elevation in pulse rate and probably in cardiac output and, simultaneously, there is a shorter circulation time. The increase in pulse rate does not appear to be due to mechanical causes. The increase in blood flow may be due to the specific dynamic action of the food, a product of digestion, such as a secretogogue, or to a hormone elaborated by the digestive tract.

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ACTION POTENTIALS FROM SINGLE MOTOR UNITS IN VOLUNTARY CONTRACTION

OLIVE C. SMITH

From the Laboratory of Physiology, Harvard Medical School

Received for publication March 28, 1934

In 1929 Adrian and Bronk demonstrated a more refined method than had hitherto been used for obtaining action potentials from muscle, not only in the laboratory animal, but also in voluntary contraction in man. These workers devised very small concentric electrodes which detect activity of the single motor units in a much restricted area. Such electrodes have since been used extensively in this laboratory and elsewhere in animal work, and they have also been used in man by McKinley and Berkwitz (1933) in determinations of "tonus," and by Hathaway (1932) in a study of reaction times, but so far as the writer is aware no further systematic investigation of voluntary contraction by this means has appeared. The present series of observations has been carried out on eight biceps and five triceps muscles in eight young normal individuals, four men and four women.

Method. The electrodes are made by inserting a fine insulated copper wire of 36 μ diameter into a 25 gauge steel hypodermic needle. A "bakelite" lacquer subsequently hardened by baking, holds the wire firmly and secures the insulation, and finally both the steel shaft and the copper point, shaved off to suit the bevel of the needle, are silver plated. The device may be sterilized by autoclaving. The butt of the needle is made to fit a small holder in such a way that the central wire leads to the grid of a three-stage condenser-coupled amplifier,[1] and the shaft is connected to ground. A Cambridge string galvanometer and an electrocardiograph camera were used to make permanent records at any desired moment, while the investigator listened to the discharge through ear phones from the amplifier.

The subject sat with the wrist hanging in a sling attached to a spring balance, in such a position that the forearm was at rest approximately at right angles to the arm. Resting position on the scale varied with the weight of the forearm and hand of the individual. Contraction in the biceps was obtained by lifting the forearm from resting position to zero position on the scale, at which point the forearm was being held, still ap-

[1] Designed and constructed by L. Garceau of this laboratory.

proximately at right angles, completely without support. Contraction of the triceps was recorded simply as pressure downward. The arrangement allowed considerable lateral play of the arm as a whole, and also the degree of rotation at the wrist was not fixed. These two factors are important in bringing in and dropping out particular motor units, and therefore the degree of contraction as measured on the scale was by no means the only determinant of the activity of a given unit. Since there is no means, in any case, of measuring the tension of the muscle fibers whose action potentials are being recorded, the scale was used merely as a rough gauge to help the subject in holding a given pose. Only minimal and submaximal degrees of contraction were dealt with, for only when a few units are being recorded can individual frequencies be distinguished. Tension was largely regulated in accordance with what was heard in the phones.

Two main questions were studied: the frequency of impulses during in-

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Action potentials of single motor units in voluntary contraction with three-stage, condenser-coupled amplifier and string galvanometer. String aperiodic. All figures except 3, 4 and 5 are retouched with white ink for reproduction.

Figs. 1 and 2. M. L., male, age 20. April 27, 1933. Time marker in 0.02 second. Sensitivity, 3 mm. = 10 μv. $\times$ 5/7.

1. Onset of contraction. Large waves, 4 in 1.18 seconds, with intervals of 0.29, 0.46 and 0.43 second, and followed by 1 second of silence (not completely shown in figure). Small waves, 8 to 9/second.

2. Another onset of contraction. Large waves, "random" for 1.24 seconds, followed by 4 seconds of silence (not shown in figure). Small waves, 13 to 14/second.

Figs. 3 and 4. M. C., female, age 27. May 13, 1933. Time as in figure 1. Sensitivity figure 3, 3 mm. = 10 μv; figure 4, 1.5 mm. = 10 μv. $\times$ 5/7.

3. Steady tension showing one unit alone, rate 12 to 13/second.

4. Steady tension; two units both at rate of 13 /second, showing synchronization for 6 consecutive beats which is most unusual.

Fig. 5. E. L., female, age 25. April 11, 1933. Time, 1 second between arrows. $\times$ 7/9. Steady tension showing a cycle of two waves passing each other, with one synchronous beat. Large waves, 7/second; small, 8.5/second.

Figs. 6, 7, 8. L. M., male, age 22. May 9, 1933. Time marker in 0.02 second shown in 8. Sensitivity, 5.5 = 10 μv. $\times$ 5/7. Increasing contraction.

6. Onset, main waves, 8/second; small waves in background.

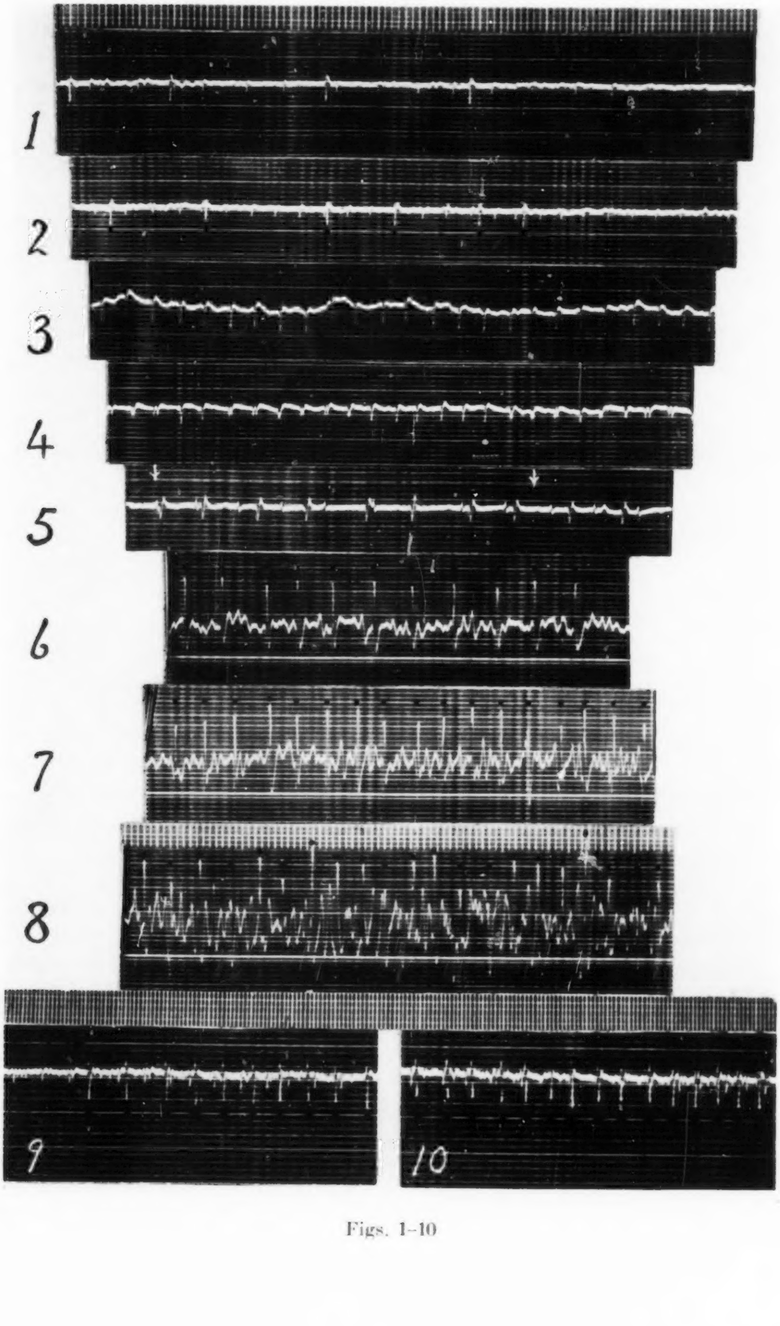
7. Two seconds later. Main waves, 11/second; background increased in amplitude.

8. Height of contraction 5.5 seconds after figure 7. Main waves, 13/second; background greatly increased in amplitude and complexity.

Figs. 9 and 10. J. R., female, age 28. March 28, 1933. Time marker in 0.01 second. Sensitivity, 1.5 = 10 μv. $\times$ 1. Increasing contraction.

9. Small wave (dotted) is the second to come in and has reached a frequency of 16/second. Onset of large wave at 10/second for 2 beats with rapid increase in frequency to 15/second.

10. Continuation 0.4 second later showing highest frequency distinguishable, 19 to 20/second, in both units at extreme right. One perfectly synchronized beat; four showing varying degrees of interference.



Figs. 1–10

crease and decrease of tension, and the fatigue of a single unit. Change of tension was carried out in two ways: *a*, by continuous movement, with continuous exposure on the film, and *b*, by discontinuous increments and decrements, giving time for exposure at each successive stage. In the trials of endurance of one unit the subject held a given position on the scale for 20 to 30 minutes while the investigator listened throughout the performance, exposures being made at one minute intervals.

RESULTS. *Increase and decrease of tension.* In general the findings corroborated those reported by Adrian and Bronk (1929) while a few supplementary points of interest were noted. With the arm completely at rest, no action potentials were either heard in the phones or registered on the film. In most cases this inactivity was easily obtained, but in one case complete relaxation had to be learned and was accomplished only after several trials. With beginning contraction in both biceps and triceps there is usually heard a faint "distant" discharge in which the ear does not distinguish a single unit, but there appears on the film one repetitive, distinct wave of small amplitude—the record of a single unit at a distance. With increase in tension the first unit increases in frequency until louder second and third ones come in and accelerate in turn (figs. 9 and 10), by which time the first one is too obscured on the film for its frequency to be distinguished. The larger waves may remain clear throughout if the contraction is not too great (fig. 8).

As a rule the frequency at the onset of each new unit is lower than the simultaneous rates of those already present, but this is not always true. Units act with considerable independence and may occasionally change their ratio to each other. But such change is probably due to slight difference in the twist of wrist or shoulder as described above, with resultant difference in the degree of activity of individual muscle fibers. This is probably also the explanation of occasional wide variation of threshold position on different trials, which occurred in two subjects.

Cessation of discharge at the end of relaxation may take place: *a*, gradually with increasing intervals in the last two or three beats, or *b*, abruptly after reaching a given slow rate, or *c*, by the dropping out of impulses in a very irregular way. It is evident that the subject's control of relaxation is very variable, and in this connection an investigation of highly trained muscle as contrasted with pathological conditions of various kinds would be of special interest.

Highest frequencies. The highest frequencies which can be clearly singled out are from 19 to 20 per second in one triceps (figs. 9 and 10), where a step-like onset of different units is also seen. Although the present investigation does not deal with frequencies in maximal contraction, it offers some evidence of the relative importance of accession of new units as compared with acceleration of frequencies. In the records of one bi-

ceps taken on two different days with two different hypodermic needles, one or two single units stand out in sharp contrast to a background which presents the appearance of the classical type of electromyogram, and this affords the unusual opportunity of following one unit amongst the "primary" and "secondary" waves and *Nebenzacken* so long discussed. Figures 6, 7 and 8 show the single unit increasing in frequency from 8+ per second to 12–13 per second at the height of contraction for that trial, while the background waves increase greatly in amplitude and only very slightly, if at all, in frequency. This is interpreted as an accession of many new units with very moderate acceleration of the single outstanding one. In another trial there is slight progressive deceleration of the single unit for four successive increments of tension while the background increases in amplitude, and subsequent increase or decrease of these two factors irrespective of each other. For one unit to diminish in activity while others increase may or may not be unusual—this was the only clear instance which occurred.[2] It is evident, however, that increase in frequency and accession of new units are two very independent phenomena.

Lowest frequencies. Low frequencies as seen in the records of 6 biceps and 2 triceps, either in steady tension, or at threshold in increase-decrease trials, range for the most part between 5 and 7 per second. In evaluating such frequencies the arbitrary criterion has been taken of a discharge of 4 or more impulses occurring without either definite change to a different rhythm, or change to such total irregularity as to make the term "rhythm" meaningless. Such extreme irregularities may be designated as "random" discharges.

The lowest frequencies under this evaluation are in one case $4\frac{1}{2}$ per second for 1.24 seconds followed by an interval equal to double the average interval in the foregoing rate; in another subject (fig. 1), four discharges at intervals of 0.29, 0.46 and 0.43 occur in 1.18 seconds followed by silence for the remaining 1 second on the film. This frequency of $2\frac{1}{2}$ per second is so very exceptional that it is a question whether it may best be termed a low frequency or a "random" discharge.

"Random" discharges occur often in a way that cannot be accounted for merely on the hypothesis of dropped beats; e.g., a single beat at onset of contraction with succeeding silence for 1 second before establishment of a rhythm. Another beginning contraction shows 6 totally irregular discharges in 1.24 seconds (fig. 2), silence for 4 seconds, and then two beats before onset at a rate of 7 per second. Such irregularity is often found also at the end of relaxation. In general it may be said that at threshold a unit may discharge in a quite random way without establishing any rhythm at all.

[2] Dr. D. B. Lindsley now working in this laboratory finds indications that this may often be true.

Fatigue. Four satisfactory experiments on fatigue of a single unit were obtained in the biceps of one man and three women. The subject, sitting in as comfortable a position as possible, flexed the arm to a point at which loud single discharges were prominent, and the attempt was made to maintain the discharge, as heard in the phones by the investigator, over a period of 20 to 30 minutes, whether or not this entailed fluctuations on the scale of the spring balance.

In L. M. a loud clear unit was heard continuously and is recorded on the film at a rate of 9± per second up to 3 minutes during a gradual change of position from 600 to 200 on the scale (cf. p. 629). It dropped out of the discharge as heard in the phones for a moment just before 3 minutes and reappeared immediately on shifting to position 150, frequency 7–8. At five minutes it stopped again momentarily and came in at position 100, rate 6 ±; it then persisted with fluctuations in frequency, up to 18 minutes. In the record taken at 11 minutes, a second, slightly larger unit (nearer the electrodes), appears on the film and the frequencies of both are accelerated; the original at this point is 12–14 per second. At 15 minutes a third appears, of about the same amplitude as the second, and all three are present to the end; the original varies in frequency from 10 to 12 per second in the last eight exposures. The acquisition of new units is, then, not a *substitution* of units, but an indication of increased activity in the vicinity of the electrodes. The "patient's" subjective feelings were noted during the latter part of the time as "general tiredness through the arm, pectoral muscles and whole shoulder, with a crick in the neck." The important finding is that the original unit has discharged at varying rates for 18 minutes with two momentary stops in the early stages and *without stop for 13 minutes.* After 15 minutes' rest it came in alone at position 900 on the scale showing the same form and amplitude as before. The change in the threshold position is not significant, as such changes were frequent in this subject.

In the next two subjects, C. B. and M. C., an individual unit was kept going for 21 and 30 minutes respectively with two momentary stops in the former case and four in the latter. In C. B. other units are present throughout the record and take a prominent part in some exposures, while in M. C. the main unit is present alone in the exposures at 7, 11 and 12 minutes and in the rest of the film the number varies from 2 to 4 irrespective of scale position. In this experiment a "momentary" pause was caught by the camera and shows a duration of 1.1 second.

In the fourth experiment, E. L. maintained approximately the same scale position for 22 minutes with a slight tendency to fluctuate toward the end. The film shows frequencies of the main unit for the most part within a range of 7 to 10 per second, but occasionally dropping to 5 or 6 and twice, to "random." Small (distant) units are present in the back-

ground and at times a second large (near) unit takes part. The longest consecutive discharge of the original was 14 minutes without stop either in the phones or on the film.

Units have been kept discharging, then, for 18 to 30 minutes with momentary stops which could hardly be regarded as recovery periods. There is no evidence in these records of substitution, or rotation of units. There may be accession of new units brought in by the effort to hold a position, but since this accession is entirely irregular and may come and go without loss of the original, it is interpreted as due to the fact noted throughout this work—that marked change in what is picked up by the electrodes may result from very delicate change in twist of wrist or shoulder. The indications are that the single unit could continue long after "fatigue" of the individual as an entity brings the test to an end.

Discussion. The present method of recording has greatly simplified the voluntary electromyogram. Adrian and Bronk (1929) have discussed in particular the findings and interpretations of Wachholder (1923) and Richter (1927) and point out that the detailed structure of the electromyogram has become clearer as the electrodes have become more selective. The complicated curves made up of "primary" and "secondary" waves and *Nebenzacken* have thereby been reduced to one type of simple discharge occurring in independent rhythms in different neuromuscular units. It is interesting that where "selection" takes place by elimination of many motor units in certain diseases of the anterior horn cells, a comparatively simple electromyogram is obtained even with plate electrodes and skin puncture (Richter and Ford, 1928), showing frequencies much in accord with present findings. Evidence appears to be all against the arguments in favor of a motor fiber discharge too high to be followed by the muscle (Forbes and Rappleye, 1917; Forbes and Olmsted, 1925) and also against a "proper" rhythm intrinsic in the motor neurone broken up by the play of centripetal impulses (v. Weizsäcker, 1921, and Dusser de Barenne and Brevée, 1926). Again, although the muscle itself may be a limiting factor in certain circumstances there appears to be no "proper" rhythm here either. Indeed the work of Adrian and Bronk (1928, 1929) finally reestablishes one contention in Piper's (1912) original thesis, viz., that the rhythm in the muscle is directly determined by the rhythm of the impulses from the central nervous system.

The question of duality of neuromuscular function has recently been brought up again by Rijlant (1933f, g) who reports two types of action potentials in striated muscle, "fast waves of high potential corresponding to contraction; much slower and smaller waves corresponding to tonic activity." He states also that such action potentials can be obtained from human triceps and quadriceps. In his original article on tonus in the rabbit (1933d), however, he says, "L'exploration électrique des muscles des

mammifères ne permet pas de mettre clairement en évidence cette dualité réactionelle si manifeste chez l'Oiseau et chez la Grenouille. Toutes les ondes observées sont rapides sans qu'il soit possible de les différencier en ondes rapides et lentes, quoique de légères différences existent entre elles." His very clear figures in this paper show that this is indeed true. But, apparently basing his hypothesis on the finding that the action potentials of greatest amplitude disappear with depression of muscular activity in light hypnosis, he maintains that the smaller action potentials which remain represent "tonic activity" as a specific neuromuscular function. The small waves disappear in deep hypnosis when the animal becomes atonic. In articles on the human electromyogram he shows figures designated as "tonus normal" (1932a, b) presenting waves of great and small amplitude of varying degrees *during this state alone*;* and in a following paper (1932c) the statement appears that in voluntary contraction in a muscle not showing tonic activity, waves are produced which are "comparable en intensité et en durée aux ondes toniques." Comparison of the figures again shows this to be true. There seem to be, then, some inconsistencies in Rijlant's interpretations. He further appears to disregard one fact in particular. The recorded amplitude is a function not only of the potential changes in the muscle fibers, but of the distance of the fibers from the electrodes. It should be noted incidentally that ordinary needle electrodes can and do pick up potentials from considerable distance (Forbes and Barbeau, 1927) and not merely from fibers in the immediate vicinity. Even coaxial electrodes, which are far more selective, record action potentials from varying distances. If, during a clear discharge, the needle is pushed slightly deeper into the muscle the discharge becomes louder (or fainter) and the simultaneous deflections of the string shadow change in amplitude, and these two phenomena are seen to be directly correlated.

In the case of the frog (1933a, b) Rijlant himself interprets the wide, slow waves as a complex made up of grouped fibrillary activities slightly asynchronous (1933f, discussion). Figures for the hen (1933c) under normal and hypnotic conditions present much the same sort of composite picture in the slow waves. The findings in the curarised cat (1933e) corroborate the known action of this drug, viz., the loss of the sustained contraction of posture before complete loss of power of active movement, but this does not offer any new proof that sustained contraction is a different function brought about by a different mechanism.

There does not appear to be in Rijlant's work any type of discharge which is not found in different degrees of sustained voluntary contraction. I believe his records demonstrate action potentials of one type varying in recorded amplitude with the distance of muscle fibers from the electrode. They demonstrate also the stretch reflex and the changes in different de-

grees of muscle tension, but they do not present any evidence of a duality of neuromuscular function.

Differently interpreted, the dropping out of large (near) units with beginning relaxation in hypnosis is a point of interest in Rijlant's work. In the present investigation it was a common finding that relatively distant units were active in small degrees of tension while the nearer ones were associated with greater contraction. This brings up the question suggested by Adrian and Bronk (1929) as to whether active fibers are more numerous in the deeper parts than in the periphery of the muscle, and is suggestive of such a possibility. There is, however, a statistical probability involved which should be considered. Since the number of units at a given distance increases with the square of the distance from the active electrode, it may be merely on these grounds that distant activity is usually the first to appear and the last to disappear. The point is one needing further investigation.

The question of rotation of activity among units suggested by Forbes (1922) to explain "tonus," or sustained contraction, in skeletal muscle without fatigue has been much discussed, but more recent work has not upheld the theory. The emphasis has all been on the surprisingly slow rates of discharge, which are in themselves sufficient to explain absence of fatigue in moderate degrees of tension. Adrian and Bronk (1929) and Denny-Brown (1929) find no indication of rotation in the single unit rhythm. The findings in the present work militate further against it. Units have been kept going without stop for 13 and 14 minutes which are periods beyond all consideration in Forbes's original idea. The momentary pauses described are hardly an indication of fatigue even at these points and neither can the appearance of other units during a 20 to 30 minute experiment be considered a rotation, since these accessions occur without loss of the original. The fact that units at very low frequencies maintain a sustained contraction *by virtue of their independence* is in a way, however, a modification of the idea of rotation in the original sense. If each unit plays its part independently the result is still an alternation of activity in succeeding fractions of time though the individual unit maintains its rhythm for long periods without cessation. It is, then, a combination of the two factors which brings about smooth moderate sustained activity in the muscle as a whole without fatigue.

SUMMARY

An investigation of action potentials in single motor units in the biceps and triceps muscles of eight young normal individuals has been made with concentric needle electrodes. The electromyogram so recorded is of simple form in moderate degrees of contraction and allows analysis of constituent rhythms.

Increase of contraction involves both increase of frequency of impulses in the individual unit and accession of new units. There is great independence of rhythm in different units.

The highest frequencies distinguishable were 19 to 20 per second. Low frequencies most often found were 5 to 7 per second, but much slower, highly irregular discharge may occur at threshold. Discharge tends to be more irregular at the end of relaxation than at onset of contraction.

In fatigue tests, individual units have maintained a continuous discharge without stop for 13 and 14 minutes, and have continued for 20 to 30 minutes with momentary pauses too short to be considered recovery periods. There is no evidence of rotation of activity, or substitution of units.

The question of duality of neuromuscular function is discussed. Since the type of discharge in moderate sustained voluntary contraction is essentially the same as that found by other investigators in "tonic" activity there is no evidence of separate "tonic" and "voluntary" mechanisms.

It is with great appreciation that I express my thanks to Dr. Alexander Forbes and Dr. Hallowell Davis for the privilege of working in this laboratory, and my indebtedness to Doctor Davis in particular, for the interest and suggestions which have formed the basis of this work.

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GLUCOSE TOLERANCE AND THE GLYCOGEN STORAGE CAPACITY OF THE DOG[1]

WINFIELD L. BUTSCH

From The Mayo Foundation, Rochester, Minnesota

Received for publication March 26, 1934

A number of observations in this laboratory on the fate of carbohydrate in the animal organism have indicated that the liver plays a predominant part in the maintenance of the normal tolerance for glucose. Question arose concerning the length of time over which an animal could assimilate glucose at its maximal rate and as to what was the maximal amount of carbohydrate that could be stored under definite, controlled conditions. When this maximum has been reached what would be the glycogen content of the liver and muscles, and would there be a decrease in the tolerance for glucose at this time? In attempting to answer these questions it is recognized that the data presented on this problem apply specifically to the conditions under which the experiments were done.

Methods and material. The experiments were all performed on healthy female dogs. These dogs previously had been trained to lie quietly in a comfortable position on a padded table for long periods. They had been on a standard kennel ration of dog biscuits, a mixture of cooked meat and cereal, and ground horse meat; food was withdrawn for twenty-four hours before an experiment, but water was permitted.

Dextrose, 10 per cent solution maintained at body temperature, was administered intravenously by continuous injection. Samples of urine were collected and measured hourly. The sugar content of the urine was determined by the method of Shaffer and Hartmann, and the phosphorus content by the method of Fiske and Subbarrow. Occasionally during an experiment, when the hourly output of urine showed signs of diminishing, it was necessary to inject from 300 cc. to 500 cc. of 0.45 per cent warm saline solution.

At the conclusion of an experiment sodium amytal (sodium iso-amyl-ethyl barbiturate) was injected intravenously to produce narcosis. Major and Bollman have shown that amytal does not appreciably decrease the glycogen content of muscle and liver under the conditions of these experiments. Duplicate samples of liver and muscle were removed and immediately placed in a freezing mixture of carbon dioxide ice and alcohol. After

[1] Work done in the Division of Experimental Medicine, The Mayo Clinic.

being thoroughly frozen, samples were weighed and immediately placed in hot potassium hydroxide solution. The amount of glycogen was determined by the method of Pflüger slightly modified. The time of intravenous injection varied in different experiments from eleven to fifty-one hours.

Experimental data. Each of four dogs (table 1) was given intravenously 3 grams of dextrose per kilogram of body weight per hour. The first point of interest is that the value for blood sugar remained at a level of from 0.30 to 0.40 per cent with a urinary excretion of from 0.5 to 0.8 gram of sugar per kilogram of body weight per hour for long periods. These periods lasted from thirty-six to fifty-one hours, at the end of which time there was an abrupt loss of the previous dextrose tolerance. The value for blood sugar rose to 0.70 or 0.80 per cent and the urinary excretion either remained at the same level or rose to values of from 1.0 to 1.5 gram of sugar per kilogram per hour. When this break in the previous tolerance occurred, the animals were killed and the glycogen content of the liver was found to be from 20 to 22 per cent, while that of the muscle varied from 2.2 to 4 per cent. Injection of one dog was continued beyond this point of break in tolerance for six hours. The glycogen content of the liver and muscle of this animal remained at the same value as that of the other dogs of the group.

TABLE 1

*Summary of results**

DOG	WEIGHT	RATE OF INJECTION	DURATION OF EXPERIMENT	DEXTROSE RETAINED	MUSCLE GLYCOGEN	LIVER GLYCOGEN
	kgm.	*grams per kgm. per hour*	*hours*	*per cent*	*per cent*	*per cent*
1	15	3	36	85.35	4.0	22
2	18	3	45	77.62		25
3	11	3	47	76.43	3.6	20
4	13	3	51	74.55	2.2	20
5	13	5	11	81.26	2.7	20
6	14.5	5	12.5	70.06	3.3	20
7	13.5	5	18	64.92	3.2	18

* This table gives the most important details of the experiments. The amount of glycogen found in the liver at the time the glucose tolerance changed was the same for several of the animals.

Each of three dogs was given 5 grams of dextrose per kilogram of body weight per hour. The value for blood sugar rose steadily until it reached 0.70 to 0.80 per cent in ten hours. The value for urinary sugar varied between 1.0 and 1.8 gram per kilogram of body weight per hour. The liver and muscle of these dogs, at the end of eleven or twelve hours, had a

glycogen content of 20 per cent and 2.5 to 3.5 per cent respectively. Injection of one dog was continued for eighteen hours. At this time there was 18 per cent glycogen in the liver and 3.2 per cent in the muscle.

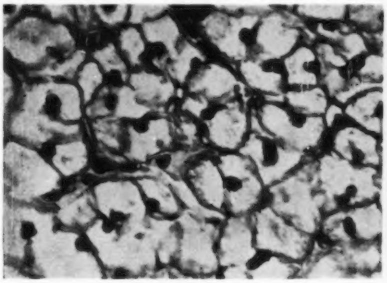

Fig. 1

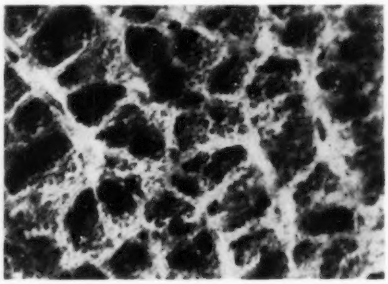

Fig. 2

Fig. 1. Photomicrograph of section of liver of animal that had been given injection of glucose when there was a break in tolerance. The section was prepared in the usual manner and stained with hematoxylin and eosin. The cells are enlarged; the nucleus is pushed to the periphery and did not stain.

Fig. 2. Photomicrograph of section of the same liver as that shown in figure 1. This section was stained to reveal the glycogen in the hepatic cells. The cells are completely filled with glycogen.

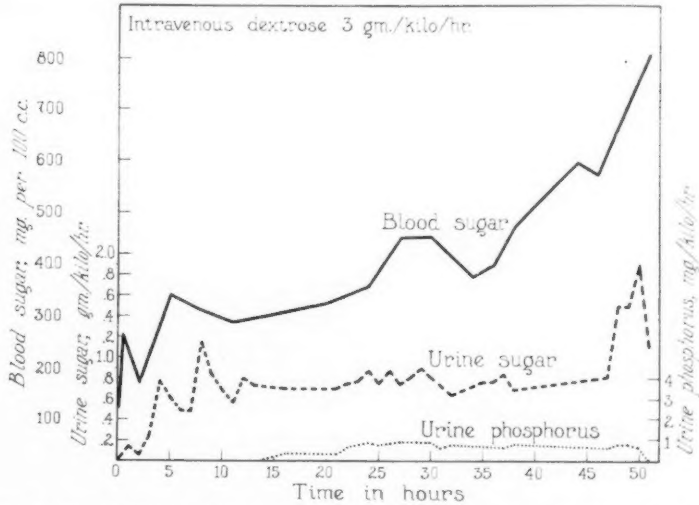

Fig. 3. The changes in blood sugar, and excretion of dextrose and phosphorus in the urine in a typical experiment. The marked rise in blood sugar and increase in excretion of dextrose at the forty-seventh hour of injection. This change in dextrose tolerance occurred at the time of maximal storage of glycogen.

With the rate of injection at 3 grams per kilogram per hour, the value for serum phosphate decreased to a trace, but recovered its original value at the end of twelve hours. The excretion of urinary phosphate was suppressed, concomitant with the decrease in serum phosphate during the first twelve hours, but phosphate was again excreted in the urine as the value for serum phosphate increased after this period of time. When 5 grams of glucose per kilogram of body weight per hour were injected, the changes in serum phosphate were the same as with the injection of 3 grams but phosphorus was not excreted in the urine.

Histologic examination of microscopic sections (made with the usual paraffin technic) of specimens of liver removed at the end of an experiment disclosed that the hepatic cells were greatly enlarged and contained clear cytoplasm that did not stain, so that only the outlines and nuclei of the cells were visible. Best's carmine stain, however, revealed that the hepatic cells were distended with glycogen which had been washed out in the usual method of fixation. All other tissues examined appeared to be essentially normal except that more granules of glycogen were present in sections of skeletal muscle (figs. 1, 2 and 3).

Comment. The results of these experiments show that the tolerance of a dog to intravenously injected glucose can be definitely and sharply broken, provided the injection is continued for a sufficient period. The point at which dextrose tolerance is broken corresponds to the maximal capacity of the animal for storage of glycogen at this particular time, for if the injection be continued beyond this point the values for glycogen are not increased. Roughly, the rate at which the storage of glycogen proceeds is dependent on the amount of dextrose presented to the tissues in a given time.

SUMMARY AND CONCLUSIONS

1. The maximal capacity of the dog's liver for storage of glycogen in these experiments was approximately 20 per cent, and the capacity of the muscle 3 to 4 per cent.
2. The dextrose tolerance of a dog is broken after this maximal capacity is reached.
3. The time necessary to reach this maximal capacity is decreased by increasing the rate of administration of glucose.

ANALYSIS OF THE FACTORS INVOLVED IN GASTRIC MOTOR INHIBITION BY FATS[1]

J. P. QUIGLEY, H. J. ZETTELMAN AND A. C. IVY

From the Department of Physiology, Western Reserve University, and the Department of Physiology and Pharmacology, Northwestern University

Received for publication March 29, 1934

The observation of Ewald and Boas (1886) that fats in the upper digestive tract decreased gastric motor activity has been repeatedly confirmed (Edelman (1906), Cohnheim (1908), von Tabora (1912), Cannon (1911), Carlson (1916)). According to the latter investigator and also Barsony and Hortobagyi (1925) reflexes over intrinsic and extrinsic nerve pathways are involved in this inhibition. A new concept was introduced when Farrell and Ivy (1926) observed inhibition of the auto-transplanted, denervated gastric pouch following introduction of fat into the main stomach (confirmed by Lim, Loo and Liu (1927), and thus established the existence of a humoral agent in gastric inhibition by fat.

Investigating the mechanism of gastric inhibition by fats, we have determined whether the phenomenon was dependent on 1, fats, soaps or glycerine which had entered the blood stream; 2, the activity of fats in augmenting the discharge of bile and pancreatic juice, or 3, the action of fats when restricted to the stomach or to the upper intestine. We have also made a more detailed study of the nature of the inhibition of denervated gastric tissue following introduction of fat into the main stomach.

Experimental. The animals employed throughout this investigation were prepared by appropriate surgical methods (described later) for the individual phases of the study and subsequently maintained in a healthy condition. They were trained to lie fully relaxed on comfortable pads during each experiment, at the beginning of which they had been fasting for twenty-four hours. The solutions for intravenous use were made in a sterile manner and were injected into the external saphenous vein immediately following their preparation, full precautions being taken to minimize all disturbing factors. Gastric motility was studied by the balloon method and a tube which passed through and for a short distance beyond the balloon was introduced into the stomach or pouch with the balloon to be used in all cases for injecting material while motility was being recorded.

a. *Does gastric inhibition follow the introduction of fat, soap or glycerine*

[1] The results of this investigation were reported at the Forty-fifth Annual Meeting of the American Physiological Society in April, 1933.

into the blood stream? For this portion of the investigation three dogs having gastric fistulae and a fourth dog with an auto-transplanted, denervated pouch prepared by the method of Ivy and Farrell (1925) were employed. In the latter animal simultaneous records were made from the pouch and stomach. Fat in the form of egg yolk emulsion was prepared after the method of Rony and Mortimer (1931). This material was examined microscopically before using and was uniformly found to be homogeneous and finely divided (one-half to two microns in diameter). The intravenous injection of such emulsions did not cause embolism, but with repeated administrations a period of less than six days between injections was necessary to avoid anaphylactic reactions. Olive oil emulsion was tried but proved to be too toxic when given intravenously for these studies. The four dogs were injected several times each (12 experiments) with 5 per cent fat (egg yolk) emulsion. Amounts ranging from 100 to 180 cc. were introduced during intervals varying from fifteen to forty-five minutes. No gastric inhibition occurred except in one experiment when loss of tone and motility (probably the result of psychic disturbance) lasted for two minutes. At the termination of these experiments, the dogs were released and offered a portion of the usual laboratory ration. In spite of the existing lipemia, the voracious manner with which the food was consumed indicated that they were experiencing *hunger*, or at least no satiation had resulted.

As much as 65 cc. of chyle containing 2.5 per cent fat, collected from the thoracic duct of a dog which had been fed egg yolks and cream, were injected intravenously (four experiments) during an interval of five to ten minutes without inhibiting gastric motility in a single instance.

The intravenous injection of 100 mgm. of chemically pure sodium oleate in 10 cc. physiological saline in five experiments was found to have no effect on gastric motility. A similar administration of 25 cc. of 5 per cent glycerine in 0.9 per cent saline solution was likewise without effect.

b. *Is gastric inhibition by fats dependent on altered gastro-intestinal secretion?* It is well established that fat ingestion leads to an increased flow of bile and pancreatic juice (involving cholecystokinin and secretin) and a decrease in gastric secretion (literature reviewed by Ivy (1930)).

The threshold dose (causing gall-bladder evacuation) of a purified preparation of cholecystokinin was established on an anesthetized dog. In fifteen experiments we subsequently determined that intravenous injection of 5 mgm. (five times the threshold quantity) did not modify spontaneous gastric motility in the normal dog.

In like manner the threshold dosage (producing a free flow of pancreatic juice) of a vasodilatin-free preparation of secretin was established and subsequently shown in fifteen experiments on normal dogs to produce no alteration in gastric motility following its intravenous administration in five times the threshold quantity.

We have not investigated the effect of factors which decrease gastric secretion but we have studied the opposite modification. The appropriate intravenous injection of liver extract was found by Kim and Ivy (1933) to produce a marked increase in gastric secretion. We employed fraction G (Cohen, Minot and Murphy), so made that 20 cc. represented 100 grams of liver and contained 4.2 grams of solids of which 0.5 mgm. were histamine-like substances. The intravenous injection of this preparation produced a slight lowering of blood pressure. When 20 cc. of fraction G were injected intravenously (3 experiments) during an interval of twenty or thirty minutes a sudden but temporary inhibition of gastric motility developed. The duration of depression was quite unlike that produced by fats administered enterally for motility returned to normal (but not above) within three to seven minutes after the beginning of the injection.

c. *The characteristics of fat inhibition of the stomach.* 1. To continue our investigation it was essential that we have detailed information regarding the typical manner in which fat administration modifies gastric motility. While making simultaneous records from the stomach and pouch in fifteen experiments performed on five dogs having auto-transplanted pouches, we injected the yolk of one egg (18 cc., 25 per cent fat) warmed to 37°C. into the stomach during an interval of one minute. For the purpose of description throughout this investigation, all time intervals are measured from the *beginning* of the injection. The typical effect of introducing one egg yolk into the stomach in one minute (fig. 1) consisted of definite depression of tone and motility of the stomach, generally beginning in one minute and complete inhibition in one and one-half minutes. Recovery of tone and motility began after 35 minutes and was complete after 40 minutes. The effect of fat on the pouch was only definite after 6 minutes when rapid loss of tone and motility began; the inhibition was complete in 7 minutes. Recovery of the pouch was evident after from 35 to 40 minutes and usually became complete at the end of 45 minutes.

2. On two occasions, motor activity was simultaneously recorded from the stomach and from the transplanted pouch preceding the second operation, at which the pedicle containing the blood vessels and nerve fibers from the celiac plexus and ganglion was cut. Introduction of the yolk of one egg into the stomach produced inhibition of both portions of gastric tissue identical with that described in C-1. If nervous impulses are involved in fat inhibition of gastric motility, they apparently are not transmitted by the nerves in the pouch pedicle.

3. In two dogs, the extrinsic nervous pathways to the stomach were eliminated by double vagotomy, double splanchnicotomy and celiac ganglionectomy. The stomach so prepared resembled the transplanted gastric pouch, but from our standpoint differed from it essentially in being periodically exposed to food and the digestive juices from the duodenum

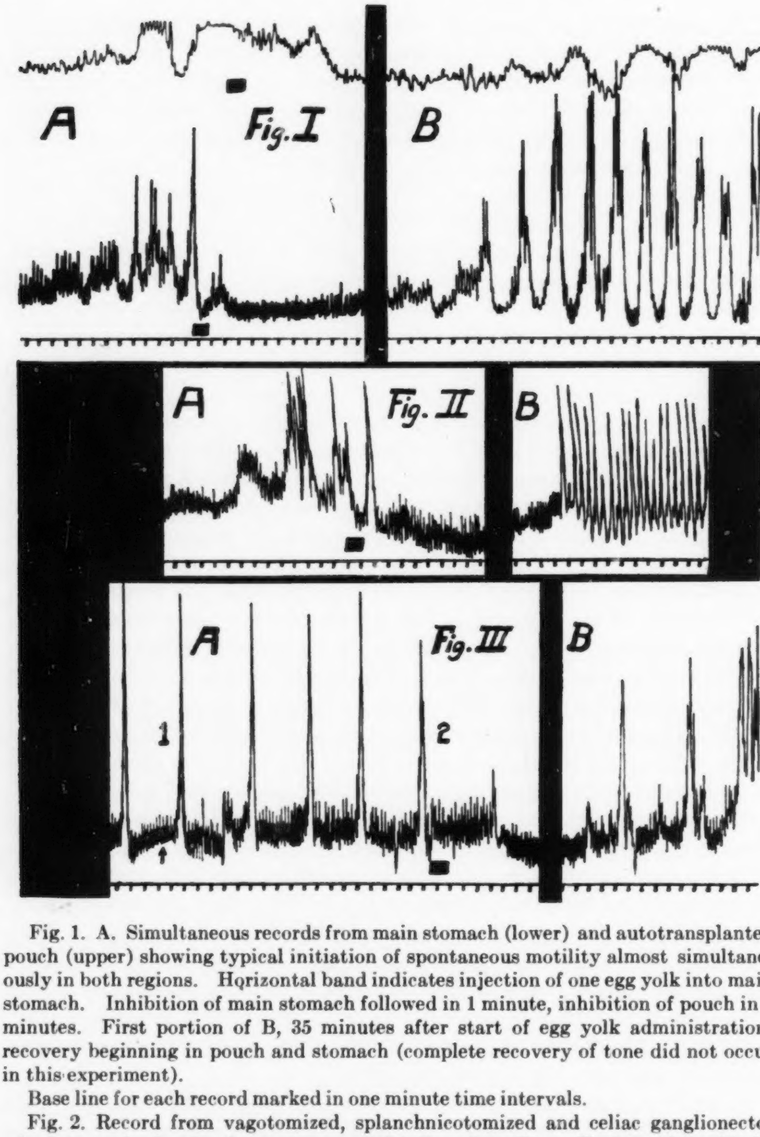

Fig. 1. A. Simultaneous records from main stomach (lower) and autotransplanted pouch (upper) showing typical initiation of spontaneous motility almost simultaneously in both regions. Horizontal band indicates injection of one egg yolk into main stomach. Inhibition of main stomach followed in 1 minute, inhibition of pouch in 6 minutes. First portion of B, 35 minutes after start of egg yolk administration; recovery beginning in pouch and stomach (complete recovery of tone did not occur in this experiment).

Base line for each record marked in one minute time intervals.

Fig. 2. Record from vagotomized, splanchnicotomized and celiac ganglionectomized stomach, showing A, spontaneous initiation of motility. Horizontal band indicates injection of one egg yolk into stomach. Gastric inhibition occurred in 3 minutes. First portion of record B, 45 minutes after beginning egg yolk administration shows return of gastric motility.

Fig. 3. Record from pouch of entire stomach. A, *1*, completion of perfusion of pouch with one and one-half egg yolk—no effect. A, *2*, introduction of one egg yolk into duodenum, gastric inhibition in 4 minutes. First portion of record B, 49 minutes after beginning of egg yolk injection, shows return of gastric motility.

and in being connected with the duodenum and esophagus anatomically and by the intrinsic nerve structures (the short or local paths). Five experiments were performed on these animals; on each occasion the yolk of one egg (as previously described) was introduced into the fasting stomach in one minute, while hunger contractions were being recorded. Complete inhibition of gastric motility and tone invariably developed (fig. 2). This inhibition differed from the typical response of the stomach having normal innervation chiefly in a slower development of depression; four minutes from the beginning of the injection was the usual latent period with this denervated stomach. Recovery was similar to that described for the normal stomach; it began in about 40 minutes and after 60 minutes was complete.

d. *The site of formation of the humoral factor and nervous factor.* The investigations on the mechanism of gastric inhibition by fat detailed by others and our experiments so far described have not determined whether the humoral factor is formed exclusively in the stomach or the upper intestine or in both regions. We have employed several methods to clarify this problem.

1. The stomachs of five dogs were separated from the remainder of the gut by a complete section at both the cardiac and pyloric sphincters. The esophagus was anastomosed to the second third of the duodenum. The cardiac end of the stomach was closed and the pyloric end brought through the abdominal wall to form an external fistula. The entire stomach pouch so obtained received some extrinsic nerve fibers (chiefly sympathetic) from the celiac-plexus along the greater and lesser curvature, but the vagi and the short nerve connections with the esophagus and duodenum were interrupted and it was no longer exposed to food, saliva or duodenal contents.

While active motility of the entire stomach pouch was being recorded, fat (egg yolk, cream or olive oil) was slowly but continuously introduced into the pouch during a 60 minute period in 8 experiments. This did not modify pouch motility. On the other hand, introduction of one egg yolk into the lower esophagus and duodenum (through a small catheter terminating in the lower esophagus) invariably produced prompt inhibition (within 3 minutes) of the pouch (fig. 3).

The above experiment was modified in one dog as follows: The pouch of the entire stomach was denervated by sectioning all blood vessels and nerves entering the lesser curvature and the remaining nerves destroyed by stripping and phenolizing the blood vessels entering the greater curvature. It was repeatedly demonstrated that introduction of egg yolk into this denervated pouch did not modify gastric motility, but the yolk of one egg introduced into the duodenum through a tube in the esophagus produced gastric inhibition within 5 minutes; normal motility returned after approximately 50 minutes.

2. The conclusion that fat in contact with gastric mucus membrane does not initiate the humoral or nervous inhibitory factors received further support from a series of experiments in which the auto-transplanted pouch was perfused as above with fat. Inhibition of the stomach or pouch resulting from this procedure was not obtained.

3. The site of formation of the inhibitory factors was more definitely established with the following preparation: A small incision was made through the serosa and muscular layers on the anterior surface of the stomach just distal to the pyloric sphincter. The submucosa and mucosal layers were cut across, and the appropriate edges invaginated by suture towards the stomach and towards the duodenum. Closure of the outer layers of the gut left a double-walled septum between stomach and duodenum but altered the nervous connections between these structures only slightly. A gastrostomy opening for free drainage of the stomach was provided and an entero-enterostomy made between the lower duodenum and upper jejunum to form a short-circuited loop about 18 cm. long. This loop was fastened to the abdominal wall and a catheter inserted into the jejunal lumen by the Witzel technic. The two animals so prepared were kept in a state of good nutrition by administration of the pabulum devised by Scott and Ivy (1931) through the catheter.

With these animals, it was repeatedly determined that fat (cream or olive oil) in the stomach did not modify gastric motility, but the slow introduction of 25 to 100 cc. of cream into the jejunum completely inhibited the stomach within 1 to 2 minutes. Complete recovery required 40 minutes or longer. It was also demonstrated that 100 cc. of Ringer's solution could be introduced into the jejunum without altering gastric motility.

Discussion. The inhibition of gastric motility by ingested fat was formerly considered to be due solely to a nervous reflex mechanism. The foregoing evidence proves that a humoral factor or mechanism is concerned, but does not show that a nervous mechanism may not also be involved in the intact animal.

The nature of the humoral agent which causes the inhibition of the motility of the extrinsically denervated gastric tissue (auto-transplanted pouch, denervated pouch of the entire stomach and the vagotomized, splanchnicotomized, celiac-ganglionectomized stomach) is not established unequivocally by our observations. However, the following observations indicate that it is a specific substance. The agent is not fat or its recognized split products, for the intravenous injection of fat (chyle or egg yolk emulsion), sodium oleate or glycerine did not inhibit. A reabsorption of bile salts occurs during fat digestion, but Still and Carlson (1929) observed only transient gastric inhibition after intravenous injection of gall-bladder bile. Secretin and cholecystokinin are produced and reabsorbed during

fat digestion, but neither they nor "gastric secretagogues" (liver extract) are the inhibitory factors (our results with purified secretin are contrary to those obtained by Tschukitschiff (1929) with crude secretin); furthermore, since Ivy and Vloedman (1923) found that histamine and "gastrin" subcutaneously does not inhibit hunger motility, apparently none of the recognized digestive hormones is involved. The extract which was obtained by Kosaka and Lim (1930) from intestinal mucosa after application of fats and which was found to inhibit gastric secretion on intravenous injection may also contain the gastric motor inhibitory agent, or the active principle in this extract may inhibit both secretion and motility.[2]

The absence of inhibition of gastric motility when fats (egg yolk, olive oil or cream) are restricted to the stomach (pouch of the entire stomach, auto-transplanted pouch or the stomach obstructed at the pylorus) demonstrates that neither the humoral nor nervous factors responsible for the inhibition are initiated in the stomach itself. The inhibitory mechanisms are initiated by fats limited in contact to duodenal or jejunal mucosa (entire stomach pouch dogs and pyloric septum dogs).

When fats are fed to a normal fasting animal, a portion evidently quickly enters the duodenum where it acts on the mucosa to liberate the specific humoral factor which may even prove to be an inhibitory autocoid, i.e., a chalone. Since the intravenous injection of chyle collected during fat absorption did not produce gastric inhibition, the humoral factor very probably does not leave the intestine in appreciable quantities through the lymph but should be sought in the blood of the intestinal veins. The short latent period indicates that natural emulsified fat (egg yolk, cream) may act as the exciting factor, at least initially, without digestion. It is possible, however, that free fatty acids (Tönnis and Never, 1925) may prove to be more potent. If a nervous factor is involved in the intact animal, the reflex is also initiated from the upper intestine by natural (undigested) emulsified fat. In either case the return of gastric motility probably involves in varying degrees a disappearance of fat from the "sensitive" portion of the intestine, temporary exhaustion of the intestinal fraction of the inhibitory agent, and decreased gastric irritability to the nervous and humoral factors.

Although fat inhibition in the types of gastric preparations studied was strikingly similar, there was an essential difference in the interval or latent period between the beginning of fat administration and onset of gastric inhibition. These intervals for the different preparations are as follows: normal stomach (normal innervation), 1 to 1½ minutes; pyloric obstructed stomach (normal innervation), 1 to 2 minutes; entire stomach pouch (short nervous paths, vagi and some sympathetics cut), 3 minutes; denervated

[2] This conclusion is supported by the report of Lim, 1933, Quart. J. Exper. Physiol., **23**, 263.

entire stomach pouch (long and short paths cut), 5 minutes; vagotomized, splanchnicotomized and celiac ganglionectomized stomach (long paths cut), 4 minutes; auto-transplanted pouch (long and short paths cut), 6 to 7 minutes; auto-transplanted pouch before cutting pedicle (some sympathetic fibers intact), 6 to 7 minutes. Since inhibition of the normal stomach occurs in $1\frac{1}{2}$ minutes, the involvement of a nervous reflex factor appears to be indicated. However, the minimal time required for production and distribution of the humoral factor is only approximately known. The latent period of denervated tissue being several times that of normal tissue may only indicate a decreased sensitivity of the former towards the humoral factor, due perhaps to absence of normal contact with food, normal innervation, normal blood supply and similar factors. If a nervous reflex mechanism is concerned in the intact animal, various types of denervation have failed to demonstrate a chief nerve path.

Since fat in the upper intestine tends to decrease gastric motility and evacuation, but increases intestinal motility (Bokai, 1888) a mechanism is provided to prevent accumulation of fat in the upper intestine (a region particularly sensitive to its presence). Abnormalities of this mechanism may occasion the nausea, eructation, regurgitation and vomiting which sometimes follow fat ingestion.

SUMMARY

Investigating the mechanism by which fats inhibit gastric motility demonstrates the absence of gastric inhibition following the intravenous injection of emulsified fat (egg yolk), or the products of fat digestion (soap, glycerine, fatty chyle). Cholecystokinin, secretin or "gastric secretagogues" (liver extract) are likewise ineffective. Initiation of the gastric inhibition is dependent on contact of fat with the mucosa of the small intestine, for the phenomenon is initiated by fat restricted to the duodenum and jejunum, but not by fat limited in contact to the stomach. Fat inhibition of gastric motility undoubtedly involves a humoral agency and the evidence strongly implies, but does not prove unequivocally that the agent is a chalone. Under normal circumstances the inhibitory process may involve both nervous and humoral mechanisms, but it is possible for the latter to act readily and completely in the absence of the former. The fat inhibition resulting exclusively from the humoral agent as indicated by the denervated or transplanted gastric pouch, differs significantly from the response of the intact stomach, chiefly in the length of the latent period which is shorter for the latter tissue.

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THE RÔLE OF CARBON DIOXIDE IN PRODUCING THE SYMPTOMS OF OXYGEN POISONING

LOUIS A. SHAW, ALBERT R. BEHNKE AND ANNE C. MESSER[1]

From the Department of Physiology, Harvard School of Public Health, Boston, Mass.

Received for publication February 12, 1934

Gesell (1923), in experiments with rats, demonstrated that oxygen at high tension increases the sensitivity of the animals to the administration of carbon dioxide. He inferred that this sensitivity was due to the disturbed elimination and transport of acid as a result of the diminished reduction of oxyhemoglobin.

Leonard Hill (1933) observed that when monkeys, goats, and rats were first exposed to 5 per cent carbon dioxide and 95 per cent oxygen, followed by an increasing oxygen pressure, the convulsions were induced more quickly or at lower oxygen pressures than in animals exposed to oxygen alone. He concluded that the increase of carbon dioxide tension in the tissues is a factor in the production of convulsions which follow exposure to high pressures of oxygen.

In the first paper of this series of articles (Behnke, Shaw, Shilling, Thomson and Messer, 1934), it was shown that the average carbon dioxide tension in the mixed venous blood of dogs breathing 100 per cent oxygen under a pressure of approximately 4 atmospheres absolute, was only 7.5 mm. higher than that found during the inspiration of air at 1 atmosphere. It seemed improbable to us that such a small increase in the carbon dioxide tension was sufficient to account for the symptoms of oxygen poisoning. Since making this statement, experimental results have been such as to lead us to infer that carbon dioxide tensions which would be entirely innocuous when associated with normal oxygen tensions, might prove toxic when associated with oxygen pressures of 4 atmospheres absolute.

From previous experiments on dogs subjected to 4 atmospheres of oxygen pressure, two well defined symptoms indicating the onset of oxygen poisoning have been observed: convulsions and a fall in blood pressure. With these symptoms as criteria of the onset of oxygen poisoning, experiments correlating the length of exposure necessary to induce the symptoms with the carbon dioxide tension in the lungs have been devised.

[1] We wish to express our appreciation of the coöperation of Mr. Robert M. Thomson who operated the pressure chamber, and of Mr. E. Preble Motley and Dr. F. S. Johnson who assisted us while under pressure.

Abnormally high alveolar carbon dioxide tensions were obtained by breathing gas mixtures enriched with carbon dioxide, and tensions below normal were produced by artificial respiration. Frequent samples of alveolar air were taken for carbon dioxide analysis.

It would be difficult, if not impossible, to carry out such experimental manoeuvres upon an animal under pressure, inclosed in a chamber just large enough to contain it, while the operator is working from the outside under atmospheric conditions. We have been fortunate in having at our disposal a compression chamber which was sufficiently large to accommodate the individuals conducting the experiments and thereby escape the disadvantages of working from the outside. In this way all the technical procedures were carried out in a manner which is essentially the same as that which is employed in the laboratory at atmospheric conditions.

METHOD. The experimental procedure was essentially the same as that described by Behnke, Shaw, Shilling, Thomson and Messer (1934). The animals used were dogs weighing between 12 and 25 kgm. Sodium diethylbarbiturate dissolved in 50 cc. of physiological salt solution was administered intraperitoneally from a syringe. The usual dose was 0.33 gram per kilogram of body weight.

The experiments were carried out in the pressure chamber described by Thomson, Yaglou and Van Woert (1932). Two of the authors remained in the chamber throughout the experiment. The partial pressure of the carbon dioxide in the alveolar air was determined by the method described by Shaw and Messer (1930). The high carbon dioxide tensions were produced by the inspiration of an oxygen-carbon dioxide gas mixture stored in a compression cylinder. The gas was allowed to flow continuously into a 6-liter spirometer, from which it was withdrawn by the dog through respiratory valves connected to a cannula inserted in the trachea. The low carbon dioxide tensions were produced by over-ventilation induced by a respirator similar to that described by Shaw and Drinker (1929), employing a respiratory rate of 20 per minute. Since the samples of alveolar air were taken at approximately 45 lb. gauge pressure and analysed at atmospheric pressure, the partial pressure of carbon dioxide in the lungs was equal to the partial pressure of carbon dioxide in the sample as determined under laboratory conditions multiplied by

$$\frac{\text{the absolute pressure in the chamber.}}{\text{atmospheric pressure}}$$

EXPERIMENTAL RESULTS. The results of our experiments are given in table 1. The carbon dioxide tension of the alveolar air appears in column 2. In experiments 4 and 5, the tension of the carbon dioxide in the lungs was calculated by adding 5 mm. to the carbon dioxide tension of the inspired air. The calculation is based upon the reduced partial pressure of the carbon di-

oxide given up to the lungs due to hyperpnea, and upon the dilution of the inspired gas by the water vapor in the lungs. A comparison of these calculations with analyses of alveolar samples shows that the error does not exceed 2 mm. In the remaining experiments, the carbon dioxide tension is derived from the direct analysis of alveolar air. The pressure of oxygen in the inspired air and the duration of the exposure are shown in columns 3 and 4. In columns 5 and 6, the onset of the fall in blood pressure and the onset of convulsions are indicated by the minutes of exposure necessary to induce these effects, or by "none" in the event that such effects fail to be induced.

The correlation between the tension of carbon dioxide in the lungs and the duration of the exposure to oxygen necessary to bring on a fall in blood

TABLE 1

Influence of the CO_2 tension in the lungs upon the fall of blood pressure and the onset of convulsions during exposure to 4 atmospheres of O_2

NUMBER OF EXPERIMENT (1)	pCO_2 IN ALVEOLAR AIR (2)	pO_2 OF GAS INSPIRED (3)	DURATION OF EXPOSURE (4)	ONSET OF FALL IN BLOOD PRESSURE* (5)	ONSET OF CONVULSIONS* (6)
	mm. Hg	*mm.* Hg	*min.*		
14	22	2986	164	None	None
11	22	3000	180	None	None
13	26	3031	175	None	None
12	27	3018	159	152	None
15	34	3059	100	79	90
8	51	3006	130	62	85
5	55†	3050	137	18	64
10	60	2935	124	20	57
4	64†	2900	78	7	78
9	68	2935	133	10	64

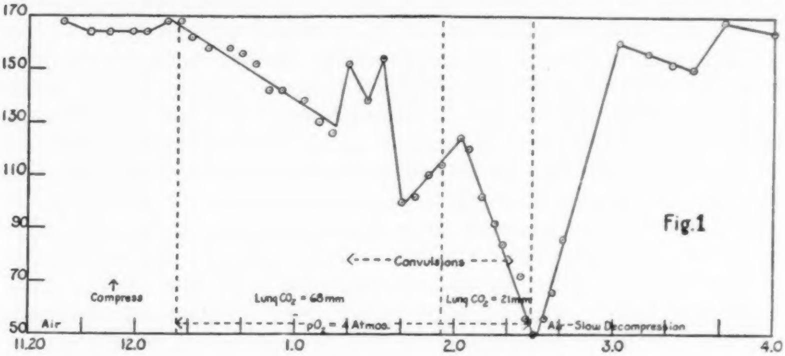
* Numbers refer to minutes of exposure.

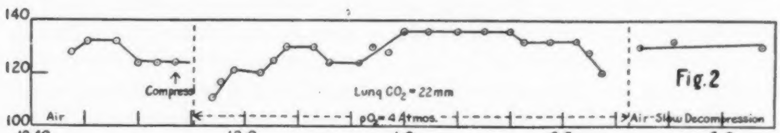
† Derived by adding 5 mm. to the pCO_2 of the inspired gas.

pressure and convulsions is clearly indicated. The higher the alveolar carbon dioxide tension, the sooner may we expect the fall in blood pressure and the onset of convulsions. On the other hand, it is apparent from experiments 12 and 15 that the symptoms of oxygen poisoning, even at subnormal carbon dioxide tensions (27 and 34 mm.), may be induced when the exposure to oxygen is sufficiently prolonged. These two experiments lead to the inference that carbon dioxide acts only as a contributing cause of the symptoms of oxygen poisoning and that the primary cause must be attributed to the toxic effect of oxygen *per se*.

Figure 1 is the blood pressure curve for experiment 9, and in all its essential characteristics is typical of the experiments in which the carbon dioxide tension in the lungs was elevated by the admixture of carbon dioxide with

the oxygen respired. At 12:16, the dog was made to inspire oxygen mixed with sufficient carbon dioxide to give a tension of approximately 60 mm. in the respired air when under a pressure of 4 atmospheres. It will be observed that the blood pressure started to fall almost immediately after the effect of the increased carbon dioxide tension was felt. At 1:20, the dog





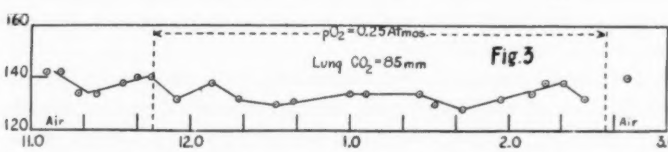


Fig. 1. Blood pressure taken during the inspiration of O_2 at 4 atmospheres of pressure combined with a high CO_2 tension in the lungs. *Ordinates*, pressure in millimeters Hg; *abscissae*, time.

Fig. 2. Blood pressure taken during the inspiration of O_2 at 4 atmospheres of pressure combined with a low CO_2 tension in the lungs. *Ordinates*, pressure in millimeters Hg; *abscissae*, time.

Fig. 3. Blood pressure taken during the inspiration of O_2 at 25 per cent of an atmosphere of pressure combined with a high CO_2 tension in the lungs. *Ordinates*, pressure in millimeters Hg; *abscissae*, time.

was seized by convulsive movements of the head, which were most conspicuous during inspiration. The violent fluctuations in the blood pressure which accompanied the convulsive period are characteristic of convulsive spasms. In experiment 8, the blood pressure fell to 80 mm. during the convulsive seizure, and in less than 8 minutes had risen to 252 mm. and

then immediately fell again. During the convulsive period in experiment 5 the blood pressure rose from 80 mm. to 186 mm., and went down again to 28 mm. in an interval of 48 minutes.

Following the onset of convulsions which may be induced by an abnormally high carbon dioxide tension in the lungs, recovery cannot be effected by the simple reduction of the alveolar carbon dioxide tension. This fact is well exemplified by the blood pressure curve in figure 1. At 1:55, pure oxygen was substituted for the oxygen-carbon dioxide gas mixture. Notwithstanding the fact that the carbon dioxide tension in the lungs was reduced to 21 mm. by artificial respiration, the blood pressure continued in its downward course to 50 mm. Death would have occurred in a few minutes had not air been substituted for oxygen. This procedure has been repeated on three other occasions with the same results. At the time that the convulsions make their appearance the respiratory rhythm is profoundly affected. After a few convulsive gasps the respiration may cease altogether or the rate may gradually fall until death follows from respiratory failure. So long as the high tension of oxygen is maintained, artificial respiration and a reduction of the alveolar carbon dioxide tension are wholly ineffectual in checking the fall in blood pressure and the onset of convulsions, once these symptoms have become well defined. In fact, a reduced oxygen pressure will bring about instant recovery even in the presence of an alveolar carbon dioxide tension which is maintained at an abnormally high level (64 mm.) by artificial means. Unless the blood pressure has been allowed to fall too low (below 50 mm.), rapid and complete recovery will invariably follow if air, even at a pressure of 4 atmospheres, be substituted for oxygen.

Figure 2 is the blood pressure curve for experiment 14, and demonstrates the well sustained blood pressure which is characteristic of low alveolar carbon dioxide tensions. After a short control period on air, the pressure was raised to 4 atmospheres and the dog breathed pure oxygen. Artificial respiration was given throughout the period of oxygen exposure at a rate of 20 respirations per minute, and of such volume as to maintain the alveolar carbon dioxide tension at about 22 mm. There was a fall in the blood pressure during the first 15 minutes of the exposure, which was probably caused by the mechanical interference with the filling of the right heart when the dog resisted the rhythmic action of the respirator. After the proper adjustment had been attained, however, the blood pressure remained at a level which departed but little from the normal value.

In presenting our observations on the blood pressure changes and the convulsions which accompany acute oxygen poisoning, it may be profitable to include our observations made upon 8 dogs which were subjects of the experiments described in the first paper of this series (Behnke, Shaw, Shilling, Thomson and Messer, 1934). The blood pressure curve of a dog

breathing oxygen at 4 atmospheres of pressure is characterized by fluctuations which are not found in the case of dogs breathing air or oxygen at 1 atmosphere, all other conditions being identical. When, under the influence of oxygen, the blood pressure falls from its normal control value, there may be a steady slow decline which is still in progress at the end of 3 hours; but usually the fall is rapid and terminates in the death of the animal in less than 1 hour from its onset. It was found that in every case in which convulsions occurred there was a preceding fall in blood pressure, and conversely we may expect that a fall in blood pressure will be followed by convulsions. In experiment 12, the fall in blood pressure was deferred until the 152nd minute of exposure, but since the experiment was terminated on the 159th minute there was hardly time for the development of convulsions. The onset of the fall in blood pressure may occur almost immediately after the exposure to oxygen or it may be deferred until the end of the experimental period. Both the time of exposure necessary to induce the symptoms of oxygen poisoning and the rate at which these symptoms bring on death are undoubtedly influenced by the tension of carbon dioxide in the lungs.

That carbon dioxide *per se* produces profound alterations in the blood pressure can be demonstrated by rebreathing experiments, in which the carbon dioxide concentration is permitted to increase while the oxygen concentration is maintained at a level somewhat above that of normal air in order to insure adequate oxygenation. To accomplish this the trachea of a dog under sodium diethylbarbiturate anesthesia was connected by valves to a 5-liter spirometer filled with oxygen. In order to prevent dilution of the rebreathing mixture with nitrogen given off from the tissues and the lungs, the dog was made to breathe pure oxygen from the spirometer for about 20 minutes while the expired air passed into the room. If this precaution is taken there is no danger of oxygen lack. The volume of the spirometer was held constant by permitting oxygen to flow in as fast as it was utilized. The oxygen concentration never fell below 40 per cent. The rebreathing period lasted from 66 to 87 minutes. Samples of alveolar air were taken at intervals and the blood pressure recorded continuously so that curves could be constructed correlating blood pressure and alveolar carbon dioxide tensions.

Three experiments of this nature were performed, the results of which are given in table 2. As the carbon dioxide concentration increased, the blood pressure started to fall very soon after the rebreathing commenced. It fell slowly and evenly for about 30 minutes when a minimum blood pressure of 100 to 114 mm. was reached, corresponding to a carbon dioxide tension in the lungs of 144 to 148 mm., at which point it again started to rise. The rise was somewhat more rapid than the fall. The peak was attained at blood pressures of 156 to 192 mm., corresponding to a carbon

dioxide tension in the lungs of 228 to 232 mm. The blood pressure fell from the peak values in about 15 minutes until complete respiratory and cardiac failure resulted in death. Though our experiments on the toxicity of carbon dioxide were not conducted in such a manner as to define the exact tension at which the blood pressure starts to fall, it is apparent that the downward trend occurs at approximately 100 mm. of carbon dioxide tension. The low and high extremes, on the other hand, are well defined and very constant.

TABLE 2

Changes in blood pressure caused by a progressive rise in the CO_2 tension of the alveolar air

NUMBER OF EXPERIMENT	NORMAL BLOOD PRESSURE	LOW		HIGH	
		Blood pressure	pCO_2	Blood pressure	pCO_2
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
1	142	100	144	192	228
2	128	112	148	156	232
3	140	114	144	156	232

O_2 pressure = 100 to 40 per cent of 1 atmosphere.

TABLE 3

The blood pressure as affected by a constant high pressure of CO_2 in the lungs

NUMBER OF EXPERIMENT	pCO_2 IN ALVEOLAR AIR	DURATION OF EXPOSURE	BLOOD PRESSURE	
			Before exposure	At end of exposure
(1)	(2)	(3)	(4)	(5)
	mm. Hg	*mm.* Hg	*mm.* Hg	*mm.* Hg
1	66*	105	144	140
2	76*	178	136	140
3	85	171	140	135
4	97	90	116	115

O_2 pressure = 25 per cent of 1 atmosphere.

* Derived by adding 5 mm. to pCO_2 of inspired gas.

Another series of experiments on dogs was done to determine whether alveolar carbon dioxide tensions of less than 100 mm. would depress the blood pressure, providing the time of exposure was sufficiently prolonged. Gas mixtures prepared in pressure cylinders containing 25 per cent oxygen, any desired carbon dioxide per cent, and the balance of nitrogen were bled into a spirometer from which the dog inspired, the expired air passing into the room, so that the carbon dioxide tension of the inspired gas was held constant. The results of these experiments are given in table 3 and figure 3. It is very significant that whereas alveolar carbon dioxide ten-

sions of 51 to 68 mm. when associated with 3000 mm. of oxygen pressure bring on a fall in the blood pressure in 62 to 7 minutes, carbon dioxide tensions of 66 to 97 mm. cause no change in blood pressure when associated with 190 mm. of oxygen pressure. In no case have any signs of convulsive movements during exposure to high carbon dioxide tensions with normal oxygen pressure been observed. These experiments show that carbon dioxide *per se* cannot be the primary cause of the symptoms of oxygen poisoning.

Discussion. The first sign of the depressant effect of oxygen becomes apparent when the blood pressure starts to fall. It is during this fall in blood pressure that the onset of convulsions may be expected. In the anesthetized dog (sodium diethylbarbiturate) the tonic and clonic movements of the extremities do not occur. The seizures are confined almost wholly to the head, neck, and thorax, and are closely associated with respiration. They are essentially inspiratory convulsions and are not unlike those in certain types of asphyxia. To these seizures we have applied the term "convulsive respiration." The convulsive respiration starts with a twitching of the face muscles and with jerky, restless movements of the head and neck. The change from rhythmical respiration to the irregular spasmodic movements of convulsive breathing is very striking. As the convulsion progresses the apneic periods between the inspiratory gasps become longer, until the convulsive period is terminated by respiratory failure. During such a seizure the blood pressure continues in its downward course, often with violent and abrupt rises of 50 mm. or more superimposed upon the curve.

Convulsive respiration and the fall in blood pressure are reversible changes which disappear rapidly when the oxygen tension is lowered. We may infer that the mechanism responsible for these symptoms is the toxic action of oxygen on the respiratory center and upon the cardiovascular centers. The circulatory mechanism continues to function, though inadequately, after the respiratory center has been completely paralyzed. The difference in the capacity of these centers to withstand the toxic effect of oxygen, however, is only a matter of time. This has been clearly shown by the fact that the blood pressure will continue to fall until death, even after the respiratory requirements have been met by the substitution of artificial respiration.

During the apneic periods which attend the convulsive seizures, exceptionally high carbon dioxide tensions in the blood have been noted. At the same time the blood pressure, which may have fallen below 100 mm. prior to the convulsion, will rapidly rise to some point above the normal level only to fall again from its high peak, ending in complete cardiac failure. The above course of events is very similar to the rise and subsequent fall in blood pressure which we have shown to take place at low oxygen pressure

after the carbon dioxide tension has exceeded a certain limit. But in appraising the relative claims of oxygen *per se* and of carbon dioxide *per se* in producing the symptoms of oxygen poisoning, we must bear in mind that although the effect of carbon dioxide upon the blood pressure when associated with high oxygen pressures is in many respects similar to the effect at low oxygen pressures, convulsions occur only when the oxygen pressure is high. Furthermore, both convulsions and a fall in blood pressure may be associated with carbon dioxide tensions which are subnormal. For these reasons it seems safe to exclude carbon dioxide as a primary cause of oxygen poisoning. The rôle which it plays is to render the oxygen more toxic or the tissues more sensitive to the effects of oxygen.

SUMMARY

1. Anesthetized dogs were subjected to an oxygen pressure of 4 atmospheres absolute, for periods of time up to 3 hours, and the effects of oxygen poisoning were observed.

2. The symptoms of acute oxygen poisoning which are most apparent are the changes in blood pressure and the convulsive seizures of the head and neck.

3. Immediate recovery from the symptoms of oxygen poisoning takes place when the oxygen pressure is reduced.

4. Death from exposure to high oxygen pressure may be caused by paralysis of either the respiratory center or the cardiovascular centers. The former is the first to cease functioning.

5. It has been shown that the higher the alveolar carbon dioxide tension the shorter the exposure to oxygen necessary to induce the symptoms of oxygen poisoning.

6. Carbon dioxide tensions which are wholly innocuous when associated with oxygen pressures of less than 1 atmosphere prove highly toxic when associated with oxygen at 4 atmospheres of pressure.

7. Carbon dioxide when combined with normal oxygen pressures does not affect the blood pressure until its tension is in excess of 100 mm., nor does it induce convulsive seizures even at a tension of 230 mm.

8. That the oxygen tension is the primary cause, and the carbon dioxide tension only a contributing cause of oxygen poisoning, is demonstrated by the fact that the symptoms may be induced by carbon dioxide tensions that are subnormal providing the exposure to oxygen is sufficiently prolonged.

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EXPERIMENTAL SODIUM LOSS ANALOGOUS TO ADRENAL INSUFFICIENCY: THE RESULTING WATER SHIFT AND SENSITIVITY TO HEMORRHAGE

ALFRED GILMAN

From the Department of Pharmacology and Toxicology, Yale University, New Haven, Connecticut

Received for publication March 29, 1934

Recent investigations on the function of the adrenal cortex have emphasized the changes in the blood stream, both physical and chemical, which result following the extirpation of these glands. Loeb, Atchley, Benedict, and Leland (1), Harrop and Weinstein (2), Harrop, Weinstein, Soffer, and Trescher (3), and Harrop, Soffer, Ellsworth, and Trescher (4) have clearly demonstrated a negative sodium balance accompanied by a marked lowering in the level of blood sodium following double adrenalectomy. Swingle, Pfiffner, Vars, and Parkins (5) have repeatedly observed that coincident with these chemical changes there is a definite hemoconcentration and lowering of blood pressure. What is more, they show that adrenalectomized dogs cannot withstand hemorrhage. The withdrawal of small amounts of blood results in a marked fall in blood pressure for which the animal is unable to compensate without the administration of cortical hormone. They conclude, "The rôle of the adrenal cortical hormone in the normal mechanism of dilution is that of a mobilizer of water and salt, i.e., it mobilizes the relatively protein free saline solutions of the tissues and interstitial spaces so that they become available for transfer to the blood stream."

Harrop, Weinstein, Soffer and Trescher (3) also have observed the hemoconcentration following adrenalectomy. They look to the urine to account for this loss of fluid from the circulation. They calculate from an estimation of the blood volume that a negative water balance of 200 cc., an amount comparable with their experimental observations, is sufficient to account for the blood concentration observed. They assume, however, that fluid lost through the urine was entirely at the expense of the blood. Presumably, all the extracellular fluids would share in this depletion, so that the blood stream would contribute only a small fraction to this negative water balance. With this fact in mind, loss of fluid by way of the kidney cannot account for the observed blood concentration. What is more, Swingle (5) denies that there is noticeable diuresis during the development of adrenal insufficiency.

One factor that has not been discussed by the above investigators is the internal shift of water which of necessity must result from a loss of sodium from the body unaccompanied by potassium. One can divide the body fluids into intracellular, containing potassium as its chief cation, and extracellular, containing sodium. The extracellular fluid may be subdivided into intra- and extravascular. Wakeman, Eisenman and Peters (6) have shown in man that the cell membrane, as characterized by the red blood corpuscle, is relatively impermeable to cations. Thus, all osmotic equilibration between the cell and its external environment must be primarily dependent upon water rather than electrolyte shift. This concept of the impermeability of cellular membranes to base may be generally applied to all cells in view of the apparent specificity of the intra and extracellular cations.

With the acceptance of this fact, what is the result of a loss of sodium in the body, bearing in mind that sodium is the cation of the extracellular fluid? As the sodium concentration, and coincidently, the osmotic pressure of the extracellular fluid falls, the cells find themselves in a hypotonic environment. In order to maintain osmotic equilibrium, there must be a passage of extracellular fluid into the cells.

This water shift resulting from a loss of sodium has been ingeniously demonstrated in vivo by Darrow and Yannet (7). In order to deplete the body of this cation, these investigators took advantage of the behavior of isotonic glucose solutions in the peritoneal cavity. When large amounts of such a solution are injected intraperitoneally, there is practically a complete equilibration of electrolytes before there is an appreciable absorption of fluid (Shecter, Carie and Darrow, 8). Thus, six hours after the intraperitoneal injection of a liter of isotonic glucose, one can withdraw from the peritoneal cavity a liter of protein free fluid having a composition almost identical with that of extracellular fluid, containing large amounts of sodium. As a result of this sodium loss into the peritoneal cavity, unaccompanied by a concomitant loss of fluid, the sodium concentration and the osmotic pressure of the blood fall markedly. At the same time, there is a shift of extracellular water into the cells, in order to maintain osmotic equilibrium. That the circulation contributes to this water shift is evidenced by an increase in serum protein and hematocrit. The swelling of the cells can be shown in the red blood corpuscles by chemical analysis.

The above blood changes, occurring within a few hours, due to the loss of sodium into the peritoneal cavity, are practically identical to those observed over the several days preceding the onset of the symptoms of adrenal insufficiency. It is the object of this paper to show that animals treated as above, and having normal adrenal function, are unable to compensate after a relatively small hemorrhage due to their altered water dis-

tribution resulting from such a Na loss, a finding duplicating that observed by Swingle in adrenalectomized animals.

Experimental procedure. Normal, healthy dogs were used as experimental subjects. A blood sample (10 cc.) was first withdrawn from the animals, after which their blood pressure was recorded by the method described by Parkins (9). The dogs then received intraperitoneally approximately 100 cc./kilo of 5.5 per cent glucose. After 150 to 300 minutes, a paracentesis was performed and the fluid withdrawn. Immediately following this procedure, the femoral artery was exposed under local anesthesia. The blood pressure of the animal was recorded. The dog was then subjected to hemorrhage and the blood pressure followed during and subsequent to hemorrhage, the first 15 cc. of blood being saved for analysis. The blood samples and the ascitic fluid were analyzed for sodium (Butler

TABLE 1

The removal of Na without fluid loss by intraperitoneal injection of glucose

DOG	WEIGHT	GLUCOSE INJECTED	FLUID RECOVERED BY PARACENTESIS	TIME BETWEEN INJECTION AND PARACENTESIS	Na CONC. ASCITIC FLUID	TOTAL Na REMOVED	Na LOST PER KILO BODY WEIGHT
	kilo	*cc.*	*cc.*	*minutes*	*M.Eq.*	*M.Eq.*	*M.Eq.*
1	13.5	1,500	1,450	180	86.0	124.7	9.2
2	12.7	1,300	1,300	150	85.2	110.7	8.7
3	9.4	1,000	950	300	98.5	93.6	9.9
4	10.4	1,000	950	300	103.2	97.9	9.4
5	14.0	1,400	1,450	300	95.5	138.5	9.9
6	12.4	1,200	1,180	300	103.5	122.1	9.9
7	12.4	1,200	1,200	180	89.9	107.9	8.7

and Tuthill, 10). Determinations of osmotic pressure (Hill, 11) and specific gravity (Barbour and Hamilton, 12) were also made.

Experimental results. *Na deprivation.* Table 1 summarizes the method used in these animals for the removal of sodium without fluid loss. As can be seen, two and one-half to five hours after the intraperitoneal injection of 100 cc. per kilo of 5.5 per cent glucose, an almost identical volume of fluid can be recovered by paracentesis.

The composition of the ascitic fluid recovered differs from that injected in that a large portion of the sugar has been replaced by sodium salts. Thus in the above experiments as much as 138.5 m. eq. of sodium was lost by one of the subjects.

Blood chemistry. Such a depletion of extracellular electrolytes, unaccompanied by a loss of water, must naturally be reflected by a change in the concentration of sodium and a lowered osmotic pressure of the extracellular fluid. Analysis of blood serum supports this contention. Table

2 depicts the marked fall in the concentration of serum sodium and osmotic pressure which occurs.

However, this loss of sodium will only be partially reflected by the fall in the sodium concentration of the extracellular fluids. The cells, in order to maintain osmotic equilibrium with their external environment, and apparently unable to lose cation through the cellular membrane, imbibe water, thus helping to maintain the sodium concentration and greatly

TABLE 2

Blood changes after Na loss into the peritoneal cavity without concomitant fluid loss

DOG	NORMAL SERUM Na	SERUM Na AFTER PARA-CENTESIS	NORMAL* SERUM OSMOTIC PRESSURE	SERUM O.P. AFTER PARA-CENTESIS	O.P. ASCITIC FLUID	NORMAL SERUM SP. GR.	SP. GR. AFTER PARA-CENTESIS
	M.Eq.	*M.Eq.*	*M.Eq.*	*M.Eq.*	*M.Eq.*		
2	149.4	136.7	162.9	151.7	154.3	1.0266	1.0386
3	145.6	127.6	154.1	146.4	159.5	1.0250	1.0348
4	141.1	126.0	149.1	134.7	147.5	1.0253	1.0325
5	145.6	129.0	154.1	145.3	156.8	1.0262	1.0375
6	149.1	127.0	153.8	140.8	152.1	1.0235	1.0330
7	148.2	133.2	152.3	145.7	157.4	1.0236	1.0349

* Osmotic pressure is expressed as M. Eq. of an isosmolar NaCl solution.

TABLE 3

Effect of altered water distribution due to loss of Na, and subsequent hemorrhage upon blood pressure

DOG	NORMAL BLOOD PRESSURE	BLOOD PRESSURE AFTER PARACENTESIS	BLOOD PRESSURE AFTER HEMORRHAGE	HEMORRHAGE	DURATION OF HEMORRHAGE
	mm. Hg	*mm. Hg*	*mm. Hg*	*cc./K.*	*minutes*
1	120	83	45	5.8	35
2	125	88	65	6.5	38
3	125	73	38	7.4	50
4	118	71	43	6.7	30
5	113	72	41	5.7	30
6	140	80	44	6.5	24
7	113	88	40	5.6	13

reducing the volume of extracellular fluid. This shift of fluid into the cell is indicated by the increase in specific gravity of the blood serum (table 2). An increase in specific gravity of this magnitude can only result from a marked change in the concentration of serum protein due to a loss of fluid into the cell. During the course of these experiments no fluid was lost through the kidney. Furthermore, Darrow and Yannet (7) have, under similar experimental conditions, demonstrated such a water shift by cellular analysis (R. B. C.).

Blood pressure changes. The effect of this fluid shift upon the blood pressure before and after hemorrhage is summarized in table 3.

The circulating volume must, of course, be reduced because of the altered water distribution of the body. A large portion of the extracellular fluid, which under normal conditions would enter the blood stream because of the high concentration of serum protein, has passed into the cell, due to stronger osmotic attraction of the higher concentration of cellular electrolytes. As a result, the blood pressure of the subjects is materially lowered as a direct consequence of this sodium loss (table 3). Hemorrhage exaggerates the above condition. With the extracellular fluid so greatly diminished, the circulatory volume bears the brunt of the burden of the loss of blood, a relatively small hemorrhage reducing the blood pressure to shock level (table 3). This is in marked contrast to the results obtained on normal dogs by Swingle (5). Such animals can withstand the loss of at least five times as much blood without appreciably affecting the blood pressure. It reproduces, however, the results obtained upon dogs suffering from adrenal insufficiency.

Treatment and recovery after hemorrhage. The obvious treatment of the above animals consists in the restoration of the lost sodium and the removal of the shifted water from the cells back to its former extracellular capacity. Inasmuch as the sodium was removed from the body unaccompanied by any water, replacement of the lost sodium with the least possible amount of a fluid vehicle would most closely approximate the original condition of the animal. Consequently, two dogs of the above series (nos. 1 and 5) were treated intravenously with 30 per cent NaCl, the amount of sodium injected approximating that lost. The results were dramatic. Immediately after the injection, the blood pressure returned to a level approximating normal, and was maintained at that level. The animal rapidly recovered from the shock symptoms evidenced after the hemorrhage, and was ambulatory within a few hours. The hypertonic NaCl was obviously effective in returning the fluid back from the cells because of the osmotic attraction of the restored concentration of extracellular sodium.

The spontaneous recovery of the animals receiving no therapy was varied. A detailed study upon a large number of dogs will have to be made before definite conclusions can be drawn. In general, it might be stated that the ability to recover is related to the severity of the treatment in respect to the amount of sodium withdrawn and the symptoms exhibited by the animals. Those subjects which showed the most severe reaction and lost the largest amounts of sodium fared very poorly. The blood pressure after hemorrhage was too low to allow adequate kidney function, a mechanism which might possibly be used to restore the blood picture to normal. They failed to recover sufficiently to voluntarily consume food, an external source of sodium to replenish that lost. The blood pressure

rose very slowly, and the animal, with such a bizarre distribution of fluid succumbed before a normal blood pressure was attained. Dogs 3 and 6 typify such behavior.

The animals which lost slightly less sodium and showed a higher serum concentration (dogs 2 and 7) were just as susceptible to hemorrhage. However, spontaneous recovery was more rapid and complete.

Discussion. The most pertinent question to be answered is, "Does the above procedure reproduce in animals the physiological picture of adrenal insufficiency as defined by our present knowledge of the changes occurring during the onset of such a deficiency?" The loss of Na from the body and the subsequent reduction in the concentration of blood sodium has been definitely established as a feature in the onset of such a deficiency by two groups of investigators. Harrop, Soffer, Ellsworth, and Trescher (4) have further stated that a negative water balance also occurs. However, this fact has been questioned by Swingle, and also above. A much greater amount of water than is observed by these authors would have to be lost to account for the marked depletion of extracellular fluid, as is evidenced by the reduction in circulatory volume. In addition, Loeb et al. have shown that there is no noticeable loss of K accompanying this negative sodium balance. Inasmuch as K is an ion rapidly excreted by the kidney, one must conclude that the cells are retaining their full amount of cation. Finally, Swingle has shown that animals in this condition are extremely sensitive to hemorrhage.

In the experimental procedure described above, these conditions were all duplicated in animals with intact adrenals. Sodium was deflected from the body without potassium and water. An internal shift of water, in order to maintain osmotic equilibrium between intra and extracellular fluids, resulted in the blood concentration and sensitivity to hemorrhage so characteristic of adrenal insufficiency. In the light of this evidence, a similar shift of water must occur after adrenalectomy, accounting for the vascular changes observed. Supporting this is the observation of Harrop (4) that after a deficient animal has been given extract, consumes food, retains sodium, and raises the concentration of blood sodium back toward its normal level, a definite diuresis is observed. If one accepts the fact that there has been a shift of water into the cell, the retention of sodium should reverse the events coincident with its loss. Thus with an increasing concentration of sodium in the extracellular fluid, water is rapidly withdrawn from the cell to become available to the kidney for excretion.

Harrop has also reported that there is no marked change in the water content of any particular tissue other than the blood. This also can be taken as evidence of an internal shift of water. In this regard, one must consider the fact that the fluid volume of every tissue can be divided into its intra and extracellular components. A shift of water from one environ-

ment to another without any appreciable water loss would be difficult to detect.

There is one marked difference in behavior in the animals described above, as contrasted to the experiments of Swingle, namely, the ability of the animals to recover after hemorrhage. After the administration of cortical hormone to his subjects, Swingle invariably reports recovery within a short period of time. In the above series of animals, the deprivation of too much sodium led to the death of the subject. Recovery in animals more mildly treated was more gradual over 24 hours. Inasmuch as all the dogs above supposedly retained adrenal function, they should correspond in their behavior to Swingle's subjects when treated with cortin. However, it is difficult to compare the two sets of experiments insofar as the amount of sodium lost is concerned. The behavior of the author's animals in respect to their recovery more closely approximates Harrop's subjects, in which case the symptoms of the deficiency were more advanced.

Another consideration is the fact that the adrenalectomized animals receive appreciable amounts of fluid intravenously with the administration of cortin. Swingle controlled this procedure by the injection of equal amounts of isotonic saline to animals in like condition. However, it must be emphasized that in such a case one is giving salt to an animal unable to retain sodium, whereas the combined administration of salt and cortin represents an entirely different type of treatment.

The treatment of adrenal deficiency in dogs by the injection of large amounts of saline has not been successful. The author's animals, suffering specifically from a Na loss, responded to an injection of hypertonic saline. However, adrenalectomized animals may suffer from other physiological defects not mentioned in this discussion (Britton, 13). The administration of isotonic saline alone to an adrenal deficient animal could only offer transitory relief, inasmuch as the sodium would again soon be lost. What is more, it would not be efficacious in the removal of fluid from the cells, a condition which also might contribute to the syndrome observed. Saline plus cortin has been proven of value, for in this case the ability to retain and concentrate sodium has been afforded. Hypertonic NaCl injection is conceivably the most rational method of salt administration. By this one procedure, the sodium concentration of the subject can be restored and the cells relieved of their excess of fluid.

Implication has been made by Swingle that cortin is capable of mobilizing fluid into the blood stream by a direct action. If the vascular syndrome observed during adrenal insufficiency is the result of sodium loss and a water shift as described above, then the depleted extracellular fluid is osmotically bound in the cell. It is difficult to see how a hormone can mobilize this water in any other way than by correcting the original physio-

logical defect. Thus, if the kidney is responsible for this sodium loss, it is logical to believe that only by renal retention of sodium and a subsequent osmotic adjustment can this condition be relieved.

SUMMARY AND CONCLUSIONS

Normal dogs were made to lose sodium without a concomitant loss of water or potassium by injection of isotonic glucose into the peritoneal cavity, and later withdrawing an equal volume of fluid by paracentesis, after ionic equilibration had taken place. This procedure resulted in a marked fall in the concentration of serum sodium and in the serum osmotic pressure. A shift of water into the cells in order to maintain osmotic equilibrium resulted in a marked depletion of the extracellular fluid, as evidenced in the circulation by an increase in serum specific gravity, and a fall in blood pressure. The subjects were extremely sensitive to hemorrhage due to this depletion of extracellular fluid, the loss of 6 to 7 cc. per kilo of blood reducing the blood pressure to shock level. The similarity between the above and adrenalectomized dogs warrants the conclusion that a similar water shift occurs in the latter animals, accounting for the circulatory phenomena observed.

Note: Since this manuscript was submitted for publication, Swingle has confirmed the observation of Harrop that dogs exhibit a negative water balance during the onset of adrenal insufficiency. However, he does not consider the water lost through the kidney sufficient to account for the extreme anhydremia observed.

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OBSERVATIONS ON THE ALTERATIONS OF BLOOD PLATELETS AS A FACTOR IN COAGULATION OF THE BLOOD

JOHN H. FERGUSON

From the Laboratory of Physiology, Yale University School of Medicine

Received for publication November 29, 1933

In pursuing the rôle of cephalin in blood coagulation, C. A. Mills (1927) demonstrated clearly that the blood platelets are devoid of prothrombin but act in virtue of their "free" and "combined" cephalin. The evidence, which resembles that of Bordet and Delange (1912), is based on the similarity of behavior of lysed platelets to purified cephalin, on the one hand, and tissue (lung) extract, on the other. The time would seem ripe for a revival of the study of the rôle of the blood platelets from the point of view of the conditions which govern their so-called lysis or disintegration by which they are enabled to exert the chemical influences noted in the above citations.

The morphological changes which accompany *in vitro* blood clotting have been reinvestigated minutely with the aid of the dark-field microscope. By controlling experimental conditions we have sought to interpret the microscopic findings. Since platelet alterations have received detailed attention in the works of very few investigators (notably Aynaud, 1909–1911; Stübel, 1914; Tait and Burke, 1926; Gichner, 1927) and are inadequately commented on by the theorists, we include a description of our own observations, which differ in some particulars from previous accounts. Accuracy of minutiae is essential to the subsequent interpretations.

Dark-field microscopy of platelet alterations during blood coagulation. A simple drop of finger prick blood is examined on a perfectly clean slide (with a coverslip) under the oil-immersion lens of a good dark-field microscope, preferably (but not essentially) at body temperature. There are no visible changes in the *erythrocytes* or *leucocytes* in the few minutes which elapse before the appearance of fibrin needles. The *blood platelets* (Zimmermann, 1860), however, display a definite sequence of morphological changes to which neither of the terms "disintegrative" and "necrobiotic" quite seem to apply. We shall simply term them "alterative." It is well known that platelet "disintegration" is essential for clotting, but, in view of the fact that certain alterations appear in anticoagulant blood platelets, our aim is to establish which type of change is prerequisite for fibrin formation. The following minutiae may be made out after some practice with the above technique:

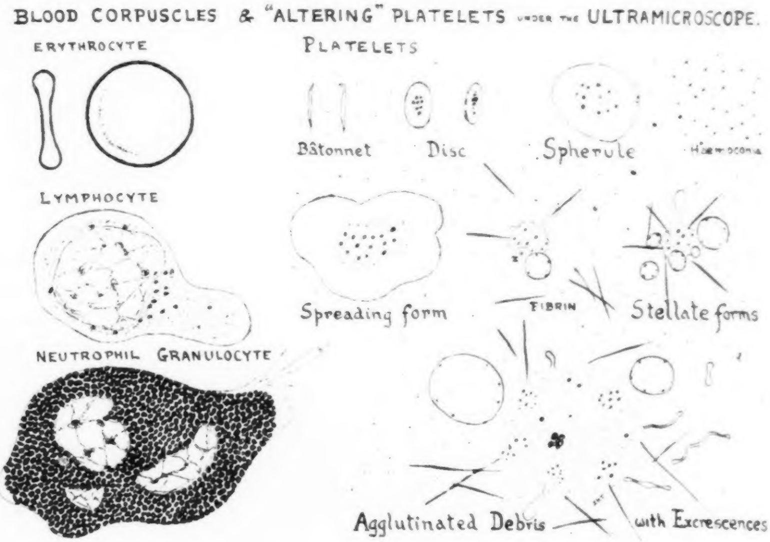


Fig. 1. Dark-field appearances of "formed elements" in coagulating human blood drop. (Oil immersion lens.)

Under optimal conditions, with the blood under observation in a matter of seconds, it is possible to identify the platelet as a free-floating ovoid *disc* of a size $\frac{1}{4}$ to $\frac{1}{2}$ that of a red blood cell. It is feebly refractile with a delicate single contour. The periphery is clear and translucent ("Optisch leer," Stübel) and in the centre lie a few refractile granules devoid of Brownian or other movement. There is no trace of a nucleus. Viewed in profile, the platelet appears "tip-cat" shaped ("bâtonnet," Aynaud) or fusiform ("spindelformig," Stübel). The only motility is the adventitious, oscillatory motion characteristic of Brownian movement. After a matter of seconds, occasionally longer, the platelet commences to alter. It first swells into a *spherule*, the shape of which may be defined when it happens to be rolling over. This form has a faint outline and a more distinct inner granulation consisting of the rather larger and brighter particles formerly observed together with a background of finer hazy granules only faintly visible. It attains to three or four times the size of the original platelet and evinces a "stickiness" which causes it to adhere to the glass coverslip, etc. Its outline may continue to expand, apparently from several foci, until a diameter of some 10μ is reached. We term this a *spreader* form. Much the more usual change, however, results in what is generally referred to as a *stellate* form, since it shows a number of processes, or, as we prefer to term

them, "excrescences," protruded from the margin of the platelet. These excrescences are of three kinds, viz., i, rounded or vesicular; ii, club-like, and iii, filamentous. The rounded type ("bosselures," Aynaud: "kugelige Bläschen," Stübel) is most frequent under these simple experimental conditions. The delicate vesicle hovers like a captive balloon at the surface of the platelet. After a certain degree of swelling it has been seen to shrink temporarily with an adjacent eddying of *hemoconia* (free particles in the plasma). It gradually swells again and its contents appear now to be fluid as judged from the vigorous Brownian movements of a few granules which may be seen to enter it from the body of the platelet. Several vesicles usually form from each platelet and their size varies up to 5 or 6 μ. Sometimes they rupture completely and disappear, but usually they remain for hours, either anchored to the residue to the platelet body or, occasionally, breaking away and floating free. The *clubs* and *filaments* are similar ex-

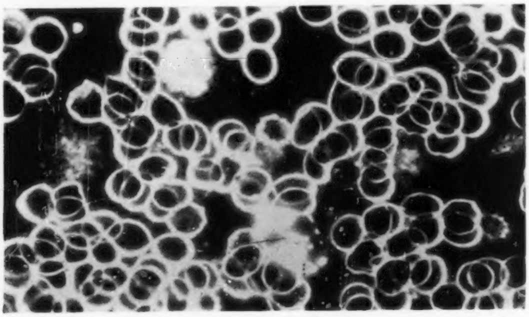


Fig. 2. Platelet "alterations" in coagulating human blood drop. Vesicular "excrescences." (Dark-field. Oil immersion lens.)

cept in shape and lack of particulate contents. They are very delicate, oscillating vigorously with the jerkiness of Brownian movement and frequently becoming detached. The feebly refractile, double contoured, free-wriggling filaments are characteristic objects. They often adhere to red cells and other objects.

From the body of the platelet, we have seen on one occasion, during the earlier phases of alteration, a bright particle jerking in and out of the parent element in a manner resembling the agonal movements of the granules of dying leucocyte fragments as described in a previous paper (Ferguson, 1930). This is depicted at x in figure 1.

The outline of the platelet body may exhibit delicate form changes for a short time after the formation of excrescences but it soon ceases to be distinguishable especially when several platelets adhere together. The ultimate result of platelet alteration is the formation of an adherent granular

matrix of particles large and small, in which body outlines have ceased to be visible. Adhering to the hazy mass are numerous vesicles, clubs, and filaments, together with many fibrin needles. (See fig. 2.)

Agglutination of platelets. This characteristic phenomenon, first described by Hayem (1878), was studied by Wright and Minot (1917) under the name "viscous metamorphosis" coined by Eberth and Schimmelbusch (1886). Under the dark-field microscope it is seen that platelets which happen to be thrown together while acquiring the "stickiness" noted during the period of swelling, readily adhere to form a clump which is increased by the addition of other platelets that chance along. It is the removal by agglutination and adherence to glass and other wettable objects, rather than disintegration, that makes it so difficult to find platelets in serum from clotted blood, and also renders platelet counts of no value if even a trace of clotting occurs.

Fibrin formation. Before fibrin is formed it is noted that the platelets must undergo alteration and agglutination. The importance of the vesicular type of excrescence formation may be emphasized in this connection. The fibrin deposit consists of fine refractile needles which appear de novo out of the dark background of the fluid plasma, the platelets, and other foreign bodies (Stübel; Tait and Burke) sometimes acting as foci for their appearance. Since many needles appear in the free plasma, the factor supplied by the altering platelets must be freely dispersed. The acicular elements grow in length and thickness during the few seconds following their appearance. As they elongate they criss-cross to form a network. After a time the refractility alters somewhat and the network acquires a more granular character with fusion nodes at the points of crossing.

Coagulation time. As a result of many comparisons with tube and other techniques for determining "coagulation time" we maintain that the time taken for the first appearance of the fibrin deposit in a simple blood drop examined with the dark field microscope (at a fixed temperature) is an excellent and ready method. It is necessary to emphasise that this "*fibrin formation time*" corresponds to the commencement of coagulation as viewed macroscopically. It will, therefore, yield shorter values than a test which depends upon the completion of coagulation in a tube of sizable diameter. We believe T. G. Perrin (1932) has recently advocated a similar technique.

Dark field observations on citrated platelets. In the technique for human transfusions by the citrate method in use in the Department of Pediatrics of the New Haven Hospital, eight volumes of blood are run from a needle in the donor's vein via rubber and glass tubing into a single volume of sterile 2.5 per cent sodium citrate solution (Parke Davis) in a corked flask with aspirating tube. The final concentration of citrate is about 0.3 per cent, so that a litre of transfusion mixture would contain not

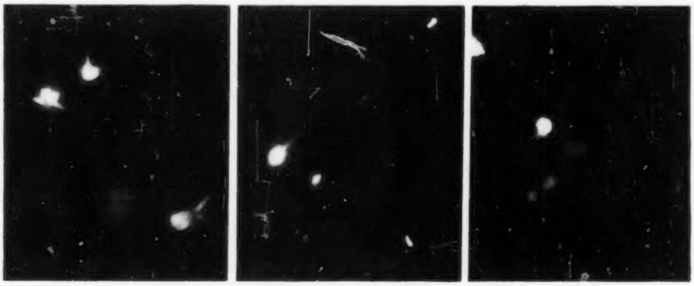
more than 3 grams of sodium citrate. We have made special investigations with these concentrations and proportions, using both rabbit heart blood and human venous blood as supplied through the courtesy of Dr. Grover F. Powers of the above Department.

Data on centrifuged plasma. *Fibrin* formation does not occur in sufficient amounts to be detected in sample drops taken for microscopic examination (dark-field) from the transfusion flask or coagulation tubes at intervals over several days. In the routine filtration of the transfusion mixture through a double thickness of sterile gauze prior to injection it is not uncommon, however, to find a small amount of fibrinous débris, especially in 24-hour specimens.

Platelet changes are greatly retarded and there are relative morphological divergences from the "normal" sequence of events. *Agglutination* of platelets is delayed for at least two hours and then occurs only to a slight extent. There is some sedimentation of the platelets to the glass slide during observation and the immobility of the sedimented forms during gross fluid currents suggests a certain amount of adherence. Both free floating and adherent platelets are rather more refractile than in ordinary blood. The tiny "disc form" preponderates at first and may be regarded as the normal. Its outline is well defined and there are a few central granules, rather coarse and devoid of Brownian or other movement. In profile it demonstrates the "bâtonnet" of Aynaud (1909). It soon begins to show delicate, short wavy filamentous protusions from one or both sides. At first these may be withdrawn and re-formed (as noted by Stübel, 1914) but in a few minutes they cease to be reversible. After 15 minutes the majority of platelets show 1, 2, 3, or occasionally, more of these filaments, which now evince a definite rigidity (fig. 3a). They are several times the length of the platelet by now, and, as time goes on, they grow larger and longer at the expense of the body of the platelet. After an hour or more the appearance is sometimes suggestive of a spermatozoon, whence we coin the term "sperm form." This type of altered platelet possesses a small ovoid body and a long, rigid, filamentous excrescence which is often curved near the middle or toward either end (fig. 3b). The central granulation becomes more distinct after the first few minutes, but apart from this change some of the platelets persist in the simple disc form for an hour or more, and a few even for days. A moderate number of platelets show the formation of a rounded type of excrescence. Seen in profile (with great ease on account of the free-floating of the majority of platelets), this is observed to be, not vesicular, but flattened, rigid and plate-like. Hence we have named it the "plaquette form" (fig. 3c). There are only one or two plaquettes developed from a single platelet, and they tend to remain small, seldom reaching the size of the platelet disc proper. They are "optically empty" and evince no granulation of fluidity of content. It is unusual for

either the filiform or plate-like excrescence to become detached (except with too vigorous manipulation) or for the platelet outline to lose its visibility. Only slight additional changes occur during the ensuing three or four days, some agglutination of the platelets being noted, together with a tendency for the excrescences to increase in size at the expense of the body of the platelet. (See fig. 3.)

DATA ON RECALCIFIED PLASMA. *Recalcification* results in a re-acceleration of the retarded clotting phenomena. The platelets become adherent and agglutinate and alter in much the usual manner. The "stellate forms" show especially well the effect of restoring the more normal in vitro conditions. From both body and filaments they quickly develop rounded excrescences which are definitely vesicular, larger, more delicate, and full of dancing granules indicative of fluid contents. The ultimate disintegration with loss of body outline proceeds in the ordinary manner. There are no



a b c

Fig. 3. Citrated blood platelets (human) dark-field microscope. (Oil-immersion lens.) a. Stellate form. b. Sperm form. c. Plaquette form.

unusual features in the deposition of fibrin. These data hold for citrated plasma recently obtained or kept for several days.

DATA WITH VARYING CONCENTRATIONS OF CITRATE. METHODS. In a series of experiments with rabbit heart blood, the blood was received into small test tubes, cleaned and rinsed out with 0.9 per cent sodium chloride immediately before use. In each tube was a definite amount of trisodium citrate (2 $Na_3C_6H_5O_7$, 11 H_2O, Baker analyzed) dissolved in distilled water to a certain strength of solution. Table 1 gives the results of a typical experiment in which the citrate solutions were mixed with five volumes of blood to give a series of final concentrations equal to one-sixth of the original strengths of solution. The data obtained include i, *clotting times*, from start to finish; ii, macroscopically visible *hemolysis*; iii, dark-field observations, with special reference to *platelet imbibition* (v. infra). The microscopic test was routinely made on the centrifuged plasma (or serum) obtained after the tubes had stood for half an hour. As a check on the

earlier phenomena in clotting specimens, sample drops were examined at intervals from the commencement of the experiments. Observations were conducted at room temperature.

TABLE 1

Rabbit heart blood mixed with varying strengths and concentrations of sodium citrate

	TUBE I	TUBE II	TUBE III	TUBE IV	TUBE V	TUBE VI	TUBE VII	TUBE VIII
Blood (cc.)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Citrate solution (cc.)	0.2*	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Strength of citrate (per cent)	0*	1.0	1.5	2.0	2.5	3.0	6.0	30.0
Final concentration (per cent)	0	0.17	0.25	0.33	0.42	0.5	1.0	5.0
Clotting time (min.):								
(a) start	2	2	30	—	—	—	—	—
(b) complete	2½	3	hrs.					
Platelet imbibition	+	+	±	—	—	—	—	—
Hemolysis	+	+	sl.	tr.	—	—	—	—

* Aq. dest. (control).
Sl. = slight; tr. = trace.

TABLE 2

Rabbit heart blood mixed with varying concentrations of "hypotonic" sodium citrate solutions

	TUBE I	TUBE II	TUBE III	TUBE IV	TUBE V	TUBE VI CONTROL	TUBE VII	TUBE VIII	TUBE IX	TUBE X	TUBE XI	TUBE XII CONTROL
Blood (cc.)	4.5	3.5	1.0	0.5	0.5	0.5	4.5	3.5	1.0	0.5	0.5	0.5
Citrate solution (cc.)	1.0	1.0	1.0	2.0	9.0	—	0.5	0.5	0.5	1.0	4.5	—
Strength of citrate (per cent)	½	½	½	½	½	—	1.0	1.0	1.0	1.0	1.0	—
Final concentration (per cent)	0.11	0.14	0.25*	0.40*	0.47*	—	0.10	0.25	0.33	0.66	0.9	—
Clotting time (min.):												
(a) start	½	4.0	del.	del.	—	4	20.0	40.0	del.	—	—	20**
(b) complete	1.0	5.5	inc.	par.		6	25.0	60.0	par.			25
Platelet imbibition	+	+	±	±	—	+	+	+	tr.	—	—	+
Hemolysis	+	+	+	+	+	—	+	+	+	+	+	—

* Gross hemolysis and great dilution of clotting factors hamper observation.
** Unusually prolonged "normal."
Del. = delayed; inc. = incomplete; par. = partial; tr. = trace.

In a second series of experiments, repeated observations were made with definite strengths of citrate used in varying proportions (to blood) with

adequate control of clotting times, etc., in untreated blood. These cover citrate solutions of definite *hypotonicity* (table 2), approximate *isotonicity*

TABLE 3

Rabbit heart blood mixed with varying concentrations of approximately "isotonic" sodium citrate solutions

	TUBE I	TUBE II	TUBE III	TUBE IV	TUBE V CONTROL	TUBE VI	TUBE VIII	TUBE VIII	TUBE IX	TUBE X CONTROL
Blood (cc.)	2.5	2.0	2.0	0.5	0.5	3.0	2.0	2.0	0.5	0.5
Citrate solution (cc.)	0.1	0.2	0.3	2.0	—	0.1	0.2	1.0	2.5	—
Strength of citrate (per cent)	2.5	2.5	2.5	2.5	—	3.0	3.0	3.0	3.0	—
Final concentration (per cent)	0.096	0.23	0.33	2.0	—	0.097	0.27	1.0	2.5	—
Clotting time (min.):										
(a) start	4.0	4.0	—	—	4.0	2.0	20.0	—	—	4
(b) complete	5.0	12.0			6.0	2.5	140.0			7
Platelet imbibition	+	+	—	—	+	+	±	—	—	+
Hemolysis	? tr.	? tr.	tr.	tr.	—	—	—	—	—	—

Tr. = trace.

TABLE 4

Rabbit blood mixed with varying concentrations of "hypertonic" sodium citrate solutions

	TUBE I	TUBE II	TUBE III	TUBE IV	TUBE V CONTROL	TUBE VI CONTROL	TUBE VII	TUBE VIII	TUBE IX	TUBE X CONTROL	TUBE XI CONTROL	TUBE XII
Blood (cc.)	5.0	2.5	1.0	0.5	0.5	0.5	10.0	4.5	2.0	0.5	0.5	2.0
Citrate solution (cc.)	0.1	0.1	0.1	4.5	—	—	0.1	0.1	2.0	—	—	2.0
Strength of citrate (per cent)	5.0	5.0	5.0	5.0	—	—	10.0	10.0	10.0	—	—	30.0
Final concentrations (per cent)	0.098	0.20	0.45	4.5	—	—	0.099	0.22	5.0	—	—	15.0
Clotting time (min.):												
Start	6.0	14.0	—	—	4.0	5.0	4.0	4.0	—	2.5	4.5	—
Complete	7.0	20.0			7.0	6.0	6.0	4.5		3.5	7.0	
Platelet imbibition	+	+	—	—	+	+	+	+	—	+	+	—
Hemolysis	—	—	—	—	—	—	—	—	—	—	—	—

(table 3) and definite *hypertonicity* (table 4). The data given are from selected typical experiments.

Analysis of data. *Prevention of coagulation* (rabbit heart blood in vitro) requires a critical final concentration of sodium citrate in the neigh-

borhood of 0.25 per cent, which is about the figure usually accepted for dog and human blood (Lewisohn). The explanation of the anticoagulant action as a depression of the calcium ion in its specific rôle is now accepted from a series of investigations of which we may recall those by Ringer and Sainsbury (1890), Arthus and Pagès (1890), Sabbatini (1900–1903) and Vines (1921).

Platelet "preservation" was noted in decalcified blood by Mosen (1893), Deetjen (1901), Bürker (1904), Aynaud (1909), Stübel (1914), Tait and Burke (1926) and Gichner (1927) et al. Our experiments show the importance of calcium ions and osmotic imbibition in the platelet alterations. The type of platelet change invariably present whenever clotting occurs may be termed "disruptive imbibition." This includes *a*, vesicular excrescence formation, and *b*, vesiculation and plasmolysis of the whole platelet (only in hypotonic solutions). In every tube in which clotting is prevented the platelets are "preserved" to the extent that the excrescences and body evince no definite fluidity of contents. The definite critical amount of citrate proves the dominant rôle of the calcium ion or *specific factor*. This is further confirmed by the fact that destructively hypotonic solutions fail to cause clotting provided that enough total concentration of citrate is present. In addition, it has been noted that citrated blood could not be be clotted by mere dilution with distilled water. The microscopic appearances, on the other hand, demonstrate that the significant platelet change is one of osmotic imbibition with excrescence formation playing a subsidiary rôle. This we may term the "*general factor*" and it is emphasized by the data on the action of various strengths of citrate solution examined for the effects of the osmotic influences, per se. In brief, *hypotonic* solutions tend to a swelling and loss of rigidity perhaps leading to complete plasmolysis of the platelet. The excrescences are especially labile and tend to be easily formed, large, numerous and very delicate. They oscillate very vigorously and often break away. *Hypertonic* solutions, on the other hand, are better platelet preservatives, judging from the minimal effective quantities of citrate. They lessen excrescence formation, increase the gel-like rigidity and tend to crumple up the body of the platelet and cause the processes to bend at bizarre angles. The analogy to erythrocyte crenation is sufficiently obvious. The *isotonic* concentration of sodium citrate may be roughly judged from a study of these diverging osmotic appearances to be rather nearer 3.0 than 2.0 per cent. After some time (minutes or hours, depending upon the disparity from isotonicity) these osmotic differences become less marked, particularly in the range which includes the isotonic zone. It may be surmised that these osmotic effects are due to a relatively slow diffusion of the citrate ion. The temporary fluid imbalance is of practical significance in clotting only if it modifies the specific lysis in which calcium ions play the dominant

rôle. The experimental data barely support this possibility since hypotonic solutions are only slightly inferior anti-coagulants at the minimum effective citrate concentration.

Citrated platelets are not "normal" platelets since they show excrescence formation, albeit in a modified manner as compared with the platelets in clotting blood. The microscopic observations of Aynaud (1909–1911) demonstrated conclusively that a careful technique, involving the use of paraffined cannula and collecting vessel and the examination of the blood in a "hanging crop preparation," could preserve the citrated platelets in the disc (and bâtonnet) form for many hours. The inference is that the primary cause of excrescence formation is the surface tension lowering action of a *wettable surface*.

A *time factor* is undoubtedly connected with platelet alteration, especially in citrated blood. In the absence of citration, excrescence formation is extremely rapid, being evident to the dark-field microscope in a matter of seconds. With citration, the process is retarded and may not commence for some minutes, or even longer in isolated platelets. In view of the rapidity of early platelet alterations and the peculiar liability of the excrescences to osmotic disturbance, it is obviously an important (and recognized) practical point to ensure citration with the utmost expedition possible. With the employment of minimal quantities of citrate in preparing a donor's blood for human transfusions, it is a technical requirement to check up on the effectiveness of citration by keeping the citrated mixture long enough to permit any partial coagulation to occur and to remove the traces of clot by straining the blood through sterile gauze before injecting into the recipient. The possible significance of platelet wetting or precoagulative alteration for the in vitro survival of transfused platelets is a pertinent question that we hope to review in another place.

Other accessory factors may include *temperature change* (*e.g.*, cooling), and *ion* (especially hydrogen ion) *imbalance* accompanying citration and the disturbance of the normal blood gas equilibria. These are at present under investigation, but we have as yet no data to suggest that they modify in any essential manner the fundamental osmotic phenomena already described.

The nature of the platelet alterations. We may dismiss the suggestion (ref. H. Deetjen, 1901, M.A. van Herwerden, 1921) that the formation of processes (excrescences) during platelet alteration constitutes a similarity with the "ameboid" behavior of leucocytes and other living cells. A more plausible idea is that of *thigmotaxis* advocated by F. Meves (1906) and J. J. Tait (1918). We have emphasised the reasons for believing that the surface tension lowering action of a wettable surface is the primary cause of platelet alteration. This leads to the formation of excrescences differing in type according to whether clotting ensues or

is prevented by depression of the calcium ion function. The fundamental conclusion of the present study is that a further change supervenes in order to liberate from the platelets the essential factor for fibrin formation. This change has been shown to be a process of osmotic imbibition and partial plasmolysis in which an essential rôle is played by the calcium ion. It is reasonable to suppose that the expanded platelet excrescence is especially liable to osmotic influences. We believe that a highly significant analogy exists in the colloidal phenomena of *myelin figure* formation from phosphatides in contact with watery electrolytes (ref. *Virchow*, 1854; *Leathes*, 1925). Leathes explains the action of various ions on myelin figure formation as specific effects on expanded molecular films. He notes that the type of lipoid is significant, a point that calls for further investigation of the lipoid chemistry of the blood platelet in view of the insistence of Howell (1912), McLean (1916) et al., that *cephalin* rather than other phosphatides plays a rôle in clotting. Further, Leathes noted incidentally that the edge of a lecithin drop in contact with calcium hydroxide became translucent. We have made dark field observations of the myelin figure formations from lecithin (Pfanstiehl) in contact with various salt solutions and note the following facts: *a*, an *initial* myelin figure formation occurs immediately with all watery solutions and is followed by a further expansion after a delay period; and *b*, the secondary phenomena are profoundly influenced by the demonstrable alternatives of i, outflow and solution of the lipoid into the watery phase, or ii, penetration of the water into the lipoid phase, the latter occurring in the presence of calcium ions, which explains the "translucent border" noted by Leathes. Thus, the ions present determine the particular phase relations of the lipoid: water dispersal. This is fully appreciated by physical chemists and G. H. A. Clowes (1913–1916) gave reasons for believing that blood clotting (among other biological systems) depended upon a physico-chemical interaction of lipoids and calcium ions in which specific phase relations were exhibited. Vines (1921) supports this view. When it is remembered that platelets contain an abundance of lipoids (Bordet and Delange, 1912), and that 1 in 100,000 of boiled brain lipoid (Clowes) will clot recalcified plasma which has been deplateletized (Cramer and Pringle, 1913) by bougie filtration, the argument appears very plausible. It will, therefore, be appreciated that the morphological minutiae of platelet alterations, when analysed physico-chemically, give much added information as to the *modus operandi* of such factors as wetting, calcium ions, and possibly specific lipoids, the functions of which in blood clotting have not been sufficiently elucidated by studies on other lines.

SUMMARY

The alterations of platelets in clotting and citrated blood have been studied with the dark-field microscope and correlated with experimental

variations of such conditions as calcium ion content and osmotically varying citrate solutions. The conclusion is reached that "wetting" of the platelet after leaving the blood stream causes the formation of excrescences which in virtue of their surface expansion are especially liable to osmotic disturbances under the specific influence of calcium ions and perhaps of the lipoids that are presumably present in the surface of the platelet. In all cases in which clotting occurs there is microscopic evidence of osmotic imbibition and partial disruption of the platelet, typically limited to the expanded excrescences. In citrated blood the platelet is preserved and its processes modified essentially in respect to their ability to show microscopic evidence of osmotic disruption, a change which is supposedly essential for the colloidal dispersion of the lipoid factors to which recent chemical investigations attribute the rôle of the blood platelet in clotting. It is evident that the clot facilitating action of cephalin and the action of anticoagulants need further investigation from the point of view of the platelet alterations without which blood can only exceptionally be caused to undergo coagulation.

Our thanks are tendered to the Universities of Cape Town and Yale for grants and facilities to enable the prosecution of these researches.

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 108, No. 3, May, 1934

A STUDY OF REFLEXES INVOLVING THE PYLORIC SPHINCTER AND ANTRUM AND THEIR RÔLE IN GASTRIC EVACUATION

J. EARL THOMAS, J. O. CRIDER AND C. J. MOGAN

From the Department of Physiology of the Jefferson Medical College of Philadelphia

Received for publication January 27, 1934

The early studies of the emptying of the stomach elicited considerable evidence that this function is influenced by stimuli acting in the vicinity of the pylorus. Cannon (1907, 1911) correlated the older observations with his study of the difference in the rate of discharge from the stomach of different foodstuffs and concluded that the emptying of the stomach is controlled by reflexes due to acid acting in the stomach to open the pylorus and in the duodenum to close it. The present study comprises an examination of these and related reflexes by more direct methods, including an investigation of the nervous mechanisms involved, and an attempt to evaluate their influence on the emptying of the stomach.

REVIEW OF THE LITERATURE. Contraction of the pyloric sphincter resulting from mechanical irritation of the duodenum was observed by Luckhardt, Phillips and Carlson (1919). This reaction was seen by Thomas and Kuntz (1926) after division of all the extrinsic nerves but not after functional separation of the duodenum from the stomach. Stimulation of the duodenum with acid and other chemical agents had a similar effect in anesthetized dogs (Carlson and Litt, 1924). Various investigators have been unable to demonstrate opening of the pylorus due to acid in the stomach (McClure, Reynolds and Schwartz, 1920; Carlson and Litt, 1924; McSwiney and Pyrah, 1932).

Reflex inhibition of gastric motility due to acid in the duodenum has been observed in the empty stomach by Boldyreff (1904) and by Brunemeier and Carlson (1914), and in the antrum separated from the rest of the stomach, by Kirschner and Mangold (1911). Pearcy and VanLiere (1926) and Herrin, Meek and Mathews (1933) observed inhibition of the stomach during distention of various parts of the intestine. Inhibition of the human stomach due to mechanical or chemical stimulation of the duodenum has been observed by McClure, Reynolds and Schwartz (1920) and by Barsony and Egan (1925).

Preliminary reports of some of the experiments to be described have been made (Mogan and Thomas, 1931; Thomas and Mogan, 1931; Thomas, 1931; Thomas and Crider, 1933).

Methods. The experiments were performed on eighteen dogs and were of such a nature as to require no anesthesia except for the preliminary operations. With the animals under morphin-ether anesthesia permanent gastric and duodenal fistulas were made and fitted with flanged rubber cannulas. Various forms of cannulas were used, the most satisfactory designs of which are illustrated in figure 1.

If leakage around the cannulas occurred it either caused loss of the animal or subsided within the second week. After recovery from the

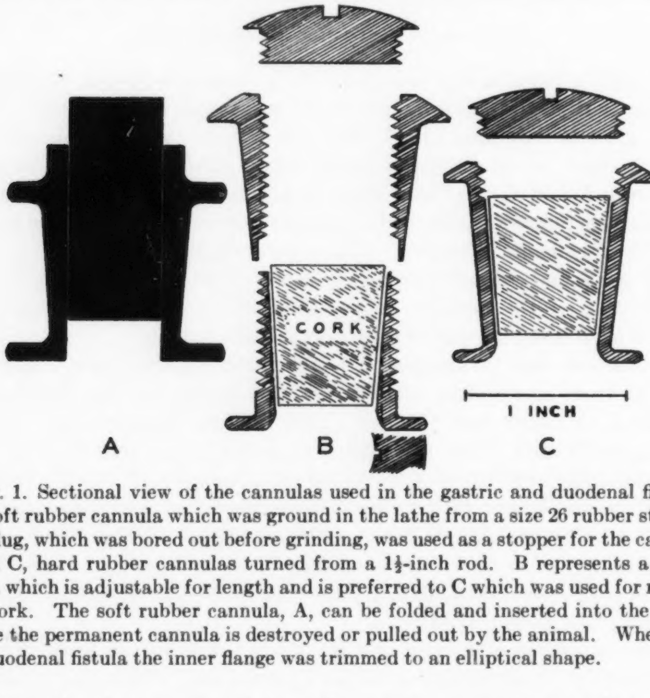

Fig. 1. Sectional view of the cannulas used in the gastric and duodenal fistulas. A, a soft rubber cannula which was ground in the lathe from a size 26 rubber stopper. The plug, which was bored out before grinding, was used as a stopper for the cannula. B and C, hard rubber cannulas turned from a 1½-inch rod. B represents a recent design which is adjustable for length and is preferred to C which was used for most of the work. The soft rubber cannula, A, can be folded and inserted into the fistula in case the permanent cannula is destroyed or pulled out by the animal. When used in a duodenal fistula the inner flange was trimmed to an elliptical shape.

operation the dogs appeared to suffer no inconvenience or discomfort; there was no evident deterioration in their general condition and some of them became quite fat. The material has been sufficient to permit of the repeated demonstration of each experimental fact on each of a series of from four to six dogs except in the study of nerve sections in which we have necessarily had to be satisfied with a smaller number.

Gastric peristalsis was recorded by the balloon method. The balloons were fixed in position in the stomach near the pyloric sphincter and were short enough to prevent their being acted upon by more than one peristaltic wave at a time. They were never inflated to such an extent that the

elasticity of the rubber entered into the development of the recorded pressure changes.

The activities of the pyloric sphincter were recorded by means of the pressure tonometer described by one of us (Thomas, 1929). This appara-

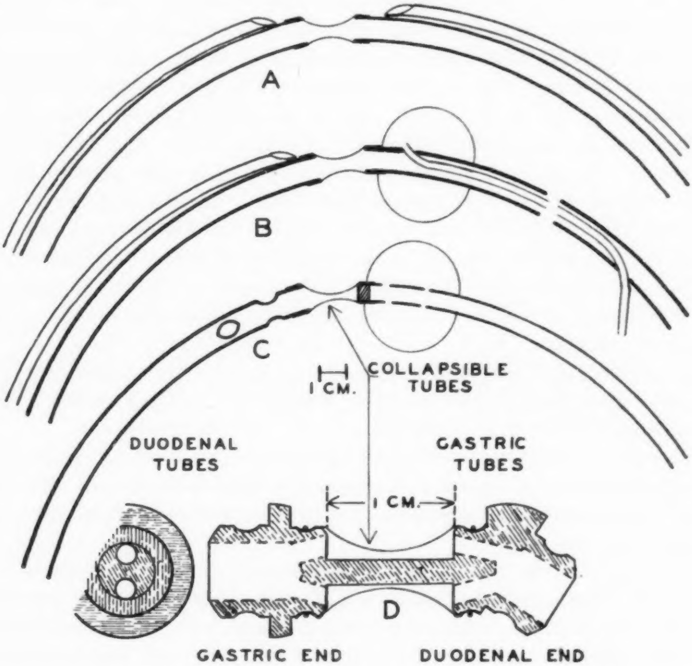

Fig. 2. Diagram showing the manner in which the tubes were arranged for the various experiments. A, gastric and duodenal tubes connected through a collapsible section at the sphincter, with injection tubes attached, used with the pressure tonometer for the study of sphincteric reflexes. B, similar to A but with the injection tube removed from the gastric side and a balloon substituted, used for simultaneous records of gastric peristalsis and sphincteric activity. C, the gastric balloon with duodenal drainage tube attached, used to study the effects of duodenal drainage on gastric motility. D, an enlarged diagram of an aluminum connector for the gastric and duodenal tubes which was used in some of the experiments on the sphincter. By serving as a rigid support for the flexible tubing under the sphincter it prevents artifacts due to kinking and also minimizes the effect of the contraction of gastric and duodenal muscle near the sphincter.

tus requires that tubes be placed, one in the stomach and one in the duodenum, the two being connected by a collapsible section which lies in the pyloric orifice. Other devices such as the gastric balloon, injection

tubes or a drainage tube were attached to these tubes or substituted for them. The several arrangements are illustrated in figure 2.

To put the tubes in place, after the animal was placed on its back and the stoppers removed from the cannulas, a woven urethral sound with an olive tip was passed through the pylorus from the duodenal side and drawn out through the gastric cannula. The tubes were fastened to one or the other end of the sound and drawn slowly through the pylorus until a sudden resistance to movement in either direction was felt, indicating that the collapsible section of the tubing was in its proper place in the pylorus. During this procedure the animals rarely exhibited any sign of discomfort or nausea. Previous experience gained by us during work on anesthetized animals enabled us to verify our judgment as to the position of the tubes from the general character of the records. In the earlier work the tubes were installed during the day preceding an experiment but this practice was abandoned when we found that apparently complete adaptation to the presence of the tubes took place generally within the first hour after they were placed.

An appropriate hammock was used to support the animal during an experiment so that it could stand or rest at ease. Many of the animals slept a considerable part of the time.

Observations were made with the stomach empty, while the animal was being fed and during digestion of a standard meal consisting of 300 or 400 grams (according to the size of the dog) of raw beef from which all visible fat had been removed.

Under the experimental conditions the animals, with few exceptions, ate their food with apparent relish, manifested vigorous gastric peristalsis and passed the gastric contents into the duodenum in a manner consistent with the usual description of these functions. The only evident disturbance of function that appeared consistently was hypertonus and hyperactivity of the pyloric sphincter but this generally subsided to a characteristic level within an hour after the tubes were placed and did not reappear unless some special maneuver involving an unusual amount of irritation was attempted.

We believe that in so far as the results are consistently reproducible, the methods are capable of yielding significant information regarding normal function. For our present purposes it is necessary only that they indicate the direction and approximate magnitude of changes in function induced by normal or artificial stimuli.

Results. *Pyloric sphincter reflexes.* For these experiments small injection tubes were attached to the air tubes used in the stomach and duodenum so that their inner ends lay within approximately 1 cm. of the sphincter on either side (fig. 2 A).

Tenth normal (0.36 per cent) HCl in 5, 10, 20 and 40 cc. amounts was

injected into the stomach when it was empty, and after feeding before the spontaneous appearance of free HCl. The results were entirely negative except that the larger injections of acid sometimes caused a moderate increase in the tonus of the sphincter.

The effect on the pyloric sphincter of acid in the duodenum was studied first on animals anesthetized with ether and morphin in order to approximate the conditions used by Carlson and Litt (1924). Under our conditions 10 or 20 cc. of N/5 HCl caused a moderate increase in the tonus of the sphincter which lasted for several minutes. Tenth normal acid was generally ineffective. The results agree, qualitatively at least, with those of Carlson and Litt.

In the unanesthetized animals the results were quite different from those observed or reported on anesthetized dogs. Immediately after the injection of acid into the duodenum the sphincter performed a series of contractions of greater frequency and amplitude than normal which generally, though not always, were accompanied by a moderate increase in tonus. This effect lasted for about one minute and was followed by a decrease in the rhythmic activity with relaxation to or below the previous tone level. This phase of the reaction lasted for several minutes and was longer and more pronounced when larger amounts of acid were injected. A typical result is illustrated in figure 3 A.

If the stomach was empty a profound effect was always produced by the first injection of 10 cc. of N/10 acid into the duodenum, and as little as 2 cc. frequently produced a detectable response. Subsequent injections were less effective. When the stomach was full 10 cc. injections frequently failed to cause an evident change in the sphincter but 20 cc. of N/10 acid were uniformly effective. In many of the weaker reactions during digestion and in some that were more pronounced there was no primary phase of contraction. Such a result is illustrated in the lower curve of figure 4.

More dilute solutions or smaller amounts of acid injected at frequent intervals either had no effect on the sphincter or caused a contraction followed by the secondary phase of quiescence. Large amounts of acid injected at intervals of a few minutes sometimes caused maintained contraction of the sphincter followed by vomiting.

The nervous mechanism of sphincter reflexes. After division of the vagus nerves in the neck (3 animals) the excitatory phase of the reaction of the sphincter to acid in the duodenum was more marked than in normal animals and the rhythmic contractions were not inhibited to the same extent; no instance of an unequivocal decrease in tonus was recorded. Qualitatively the results resembled those obtained in anesthetized animals with the nerves intact. The results are illustrated in figure 3.

No further change in the reaction was observed as a result of splanchnic section following vagus section (1 animal).

Gastric reflexes. For recording gastric peristalsis a small balloon was attached to the gastric tube so as to rest within the stomach as near as possible to the pyloric sphincter. The balloon held approximately 20 cc. of air without stretching and was inflated with 10 to 15 cc. when in use. A few experiments performed with two and three gastric balloons in series

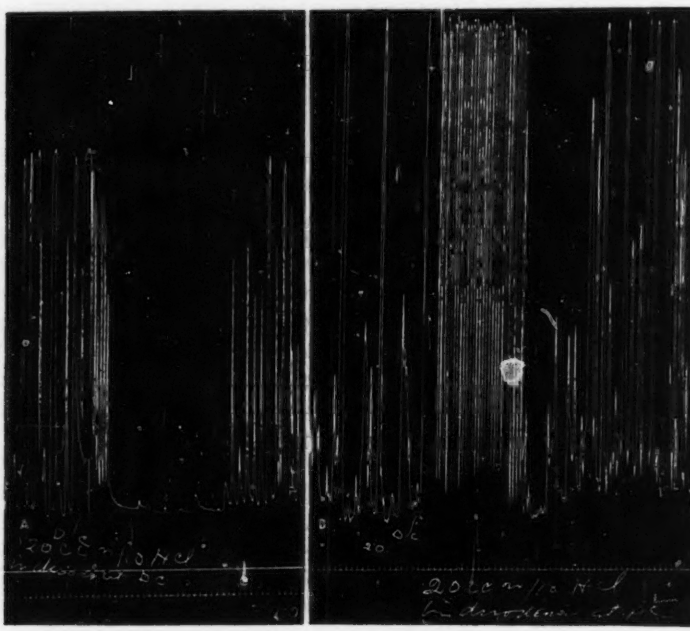

Fig. 3. Reaction of the pyloric sphincter to HCl in the duodenum (A) before, and (B) after, section of the vagus nerves in the neck. Both records are from the same animal and both made with the stomach empty. No account is to be taken of the height of the maximal contractions since this was limited in both records by the use of a safety valve in the air line. Records were made with a phosphoric acid (sp. gr. 1.7) manometer in connection with the pressure tonometer. The symbol *DC* used to mark the point of injection means "duodenum closed" and refers to the practice of draining excess fluid from the duodenum before injecting acid. Time is in 10 second intervals.

indicated that we were recording peristalsis and not merely local rhythmic contractions. If the contractions of the sphincter were to be recorded at the same time, the gastric and duodenal air tubes were used as in the experiments with the sphincter alone and a small accessory tube was placed within the gastric tube and passed through its wall, at one end into the lumen of the balloon and at the other to connect with the recording system

(fig. 2 B). In other experiments duodenal drainage was provided for by replacing the air tube in the duodenum with a tube in which several large holes had been burned near the pyloric end (fig. 2 C).

The results of Brunemeier and Carlson (1914) and others showing that

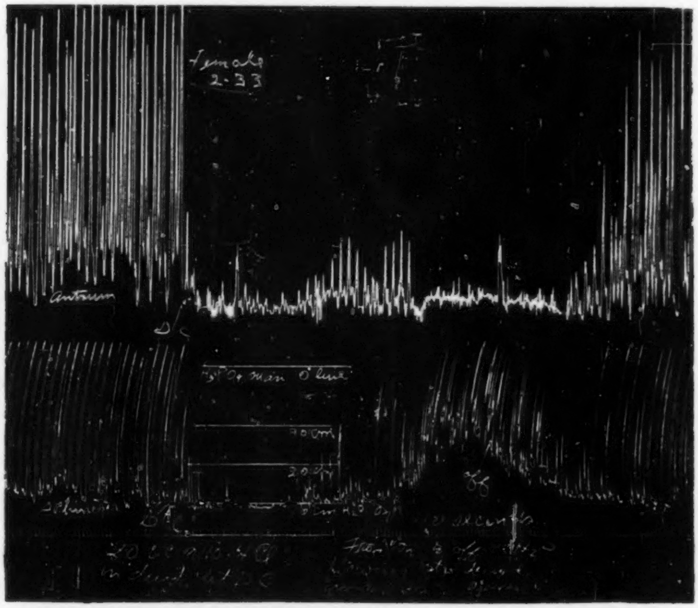

Fig. 4. Record showing the effect on the pyloric sphincter and on peristalsis of injecting acid into the duodenum during digestion. Gastric peristalsis (upper curve) was recorded with the balloon connected to a phosphoric acid (sp. gr. 1.7) manometer. The base line for this record is marked "H_3PO_4 man. O line." The sphincter record below was made with a tambour connected through a phosphoric acid manometer with the pressure tonometer. The short horizontal lines show calibration of the recording system in cm. of water pressure. A safety valve was used which limits the height of the record of the stronger contractions. The latter part of the curve is a check on the apparatus and shows, incidentally, contraction of the sphincter and inhibition of peristalsis due to mechanical stimulation (distention) of the duodenum.

acid in the duodenum inhibits the activity of the empty stomach were confirmed with respect to the pyloric portion.

We found that during digestion as well, acid and other substances in the duodenum decreased the force of gastric peristalsis in the pyloric region or, if injected in adequate amounts, stopped it completely for several minutes (fig. 4, upper graph). Besides HCl the following materials, listed

in the approximate order of their effectiveness were found to act as adequate stimuli:

Hypertonic sodium chloride solution (5 per cent)
Isotonic acid phosphate solution
Ethyl alcohol (10 and 50 per cent)
Gastric contents
Tap water
Isotonic sodium chloride solution (0.9 per cent)

The relative thresholds for sphincteric contraction and gastric inhibition. In the experiments in which simultaneous records of sphincteric and gastric activity were obtained moderate amounts of acid in the duodenum exerted a more marked influence on the motility of the stomach than on the tonus of the sphincter; for example, in many of the experiments with 10 cc. of N/10 HCl the only effect on the tone of the sphincter was a slight decrease while at the same time the peristaltic contractions (and the rhythmic contractions of the sphincter as well) were definitely diminished in amplitude. In some animals almost complete cessation of gastric peristalsis could be elicited with acid stimuli to the duodenum which failed to cause even a momentary increase in the tonus of the sphincter. Such results were obtained more frequently when the stomach was full. A typical result of this sort is illustrated in figure 4.

The influence of the normal duodenal contents. In the experiments on the effect of acid in the duodenum during gastric digestion vigorous gastric peristalsis of regular amplitude was needed in order to demonstrate the slight effect of some of the weaker stimuli. We were able to obtain these conditions for only a short time after the animal was fed. The cause of this difficulty was discovered while draining the duodenum in preparation for an injection of acid. On several such occasions we noticed that gastric peristalsis increased in amplitude and became more nearly regular. In other experiments, not connected with this study, we had noticed that after prolonged duodenal drainage large masses of meat appeared in the duodenum which, under ordinary circumstances, would probably not have left the stomach. It seemed probable that duodenal drainage removed an inhibitory influence affecting gastric peristalsis.

In order to test this hypothesis experiments were performed in which the usual procedure was to insert a gastric balloon and duodenal drainage tube (fig. 2 C) and, with the outer end of the drainage tube clamped, allow the animal to eat the standard meal. After feeding, the variable and irregular activity of the empty stomach was promptly replaced by regular peristalsis. In the pyloric portion at least, the force of the contractions as indicated by the amplitude of the balloon record was greater during the first half-hour after feeding than at any subsequent time. In control experiments in which there was no experimental interference the average

amplitude, after attaining its maximum, progressively declined throughout the period of observation; within two or three hours it was often less than half the maximum attained after the meal. Spontaneous increases in amplitude occurred from time to time but they were generally of short duration and seldom reached the height of the earlier contractions.

If at any time after the amplitude of the contractions had begun to diminish the duodenal drainage tube was opened and the duodenal contents along with any material added from the stomach drained to the outside, the contractions began to increase in amplitude and continued to increase

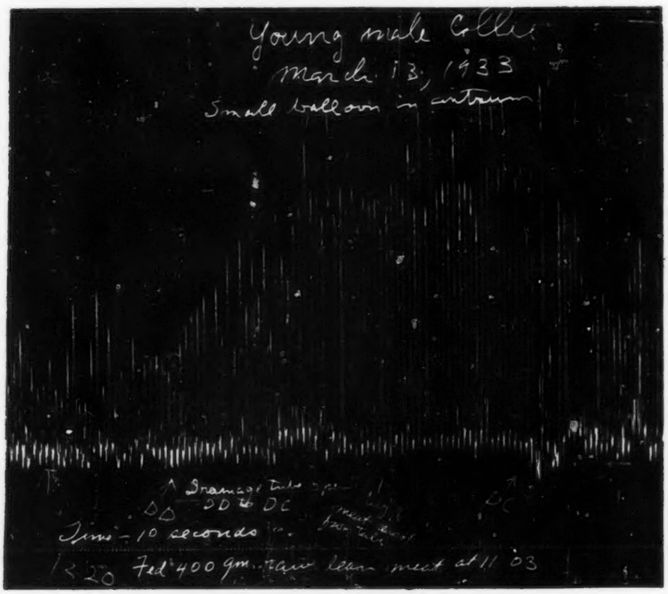

Fig. 5. Record showing the effect of duodenal drainage on gastric peristalsis. Record with phosphoric acid manometer.

as long as the drainage was continued until they reached or exceeded the original amplitude. Closing the drainage tube so that the gastric contents passed into and through the duodenum in a normal manner was followed by a gradual decrease in the amplitude to or below the level preceding the drainage. The effect of duodenal drainage is shown in figure 5, which is typical except that the ultimate decrease in amplitude following closure of the drainage tube could not be included in the short record.

The latent period for increase in amplitude following the beginning of duodenal drainage was often less than one minute but was apt to be longer

if the experiment was performed several hours after the animal was fed. There was a sufficient number of experiments in which the latent period was less than one minute to eliminate any reasonable possibility that the changes were due solely to hormones or to the presence in the blood of products of digestion. The latent period for decrease in amplitude following closure of the drainage tube was longer and more variable, extending in rare instances to thirty minutes. This would probably be influenced by the rate of emptying of the stomach and, in any case, suggests that summation of stimuli from the intestine is an important factor in the development of the inhibitory reflex.

The phenomenon was demonstrated in a most striking manner and with a very brief latent period by allowing the animal to drink water. With the duodenal drainage tube closed the drinking of water was followed, generally within less than one minute, by a decrease in the peristaltic contractions to a small fraction of their former amplitude. If the drainage tube was then opened the peristalsis regained its former amplitude almost immediately. If the drainage tube was open when the animal drank there was practically no change in the amplitude of the peristalsis but closing the tube while the fluid was leaving the stomach invariably caused prompt inhibition.

Other variations of the experiment were tried but it is unnecessary to report them inasmuch as the results were uniformly consistent with the hypothesis that the irregular and diminished force of peristalsis in the pyloric portion of the stomach which occurred constantly in the absence of experimental interference was due to the presence in the intestine of its normal content.

The nature of the stimulus for gastric inhibition. To determine whether the stimuli concerned in the normal inhibition of the stomach from the intestine were chemical or mechanical we compared the effect of alternate injections of Locke's solution and of the same amount of material previously drained from the duodenum. We had already found that rapid injection of 20 cc. of normal saline or of Locke's solution into the duodenum exerted a very brief but definite inhibitory influence on the amplitude of gastric peristalsis. The drainage material was intended to represent the material to which the first part of the duodenum is normally exposed while the stomach is emptying.

Fourteen experiments were performed on two dogs, each consisting of six injections alternating Locke's solution with drainage material. The average recorded height of the ten contractions following each injection was compared with that of the ten preceding contractions and the percentage reduction in amplitude computed. The average reduction in amplitude produced by the injections of drainage material was 31.6 per cent; the corresponding figure for Locke's solution was 9.3 per cent. The results

indicate that the stimulus due to the drainage material was in part chemical. Inhibition of peristalsis due to mechanical stimulation of the duodenum is shown in the latter part of the upper graph in figure 4.

The nervous mechanism of the gastric inhibitory reflex. Duodenal reflexes affecting gastric motility were studied on three animals after division of

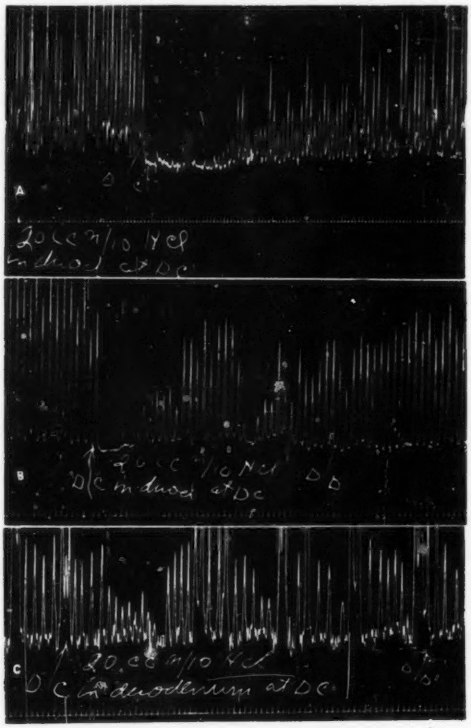

Fig. 6. Inhibition of gastric peristalsis due to acid in the duodenum; A, in the normal animal; B, after section of both splanchnic nerves; C, after section of both splanchnics and both vagi. All records from the same animal with the same apparatus (balloon and H_3PO_4 manometer) and with the stomach empty. The genuineness of the inhibition shown in the lowest graph was established by repeated observations.

both vagus nerves and on one of these after subsequent section of the splanchnic nerves. One animal was studied after section of the splanchnics, leaving the vagi intact, and later after section of the vagi also. Control experiments were performed on these animals previous to nerve section in which the major reflexes described above were demonstrated. The vagus nerves were severed in the neck without preserving the recurrent laryngal

nerves, a precaution that is not necessary in the dog. After vagus section they were fed through the gastric cannula. The splanchnics were cut through a lumbar incision and the division verified at autopsy.

Primary section of the splanchnic nerves was without effect on the reflexes studied (fig. 6 B). Likewise, division of the splanchnics subsequent to vagus section caused no further change in the reflexes. These facts constitute a clear cut difference between these reactions and the gastric inhibition due to noxious stimuli described by Cannon (1905) (1909) and by Cannon and Murphy (1907).

After vagus section stimuli of moderate intensity which were generally adequate in the normal animal were not effective. Injections into the duodenum of 10 cc. of N/20 HCl, 5 per cent NaCl or 10 per cent alcohol did not affect gastric motility. We were likewise unable to demonstrate on animals so prepared the characteristic effect of normal duodenal filling

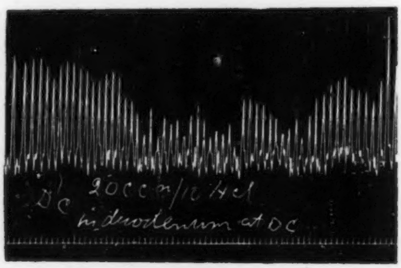

Fig. 7. Inhibition of gastric peristalsis due to acid in the duodenum after section of the vagi only (splanchnics intact). Conditions the same as described under figure 6 except that a different animal was used.

or duodenal drainage. Stronger stimuli (20 cc. of N/10 acid) produced a moderate inhibition which differed from the normal in that it had a longer latent period (approximately $1\frac{1}{2}$ min. as compared to a few seconds in the normal) and had a smaller proportional effect on the amplitude. Our results after vagus section are practically identical with those obtained by Brunemeier and Carlson (1914) after section of all the extrinsic nerves except that we found a shorter latent period than they reported, probably because we were dealing with a part of the stomach nearer the point of stimulation. Our results are illustrated in figure 7. (Compare with fig. 6 C which shows the result obtained after section of both vagi and both splanchnics.)

After vagus section the first few peristaltic contractions of the pars pylorica following the injection of acid into the duodenum frequently had a slightly greater amplitude than those preceding. That this represented an actual excitatory phase, occurring at a time when in the normal animal

gastric peristalsis would be completely inhibited, was demonstrated by the following experiment. Repeated injections of HCl were made into the duodenum, each being given just before the end of the latent period for inhibition following the preceding injection, i.e., at about $1\frac{1}{2}$ minute intervals. Each injection was followed by the usual number of strong gastric contractions so that no inhibition was apparent until the injections were stopped when it followed the last injection after the usual latent period for inhibition following a single injection.

We noted incidentally that pilocarpin in doses approximately equivalent to the human therapeutic dose (0.08 mgm. per kilo) completely abolished the residual inhibitory reflex after vagus section for a period of from four to five hours after the drug was given. A similar but much weaker effect on the inhibitory reflex in normal dogs could be elicited with somewhat larger doses of pilocarpin; the reflex was abolished for only a short time at the height of the action of the drug.

DISCUSSION. *Neurological considerations.* The evidence from the literature and from our results proves that adequate stimulation of the duodenum initiates two reflexes, one of which tends to cause contraction of the pyloric sphincter and the other causes inhibition of the stomach. The first was found not to be dependent upon the extrinsic innervation and is, evidently, the ascending excitatory component of the familiar myenteric reflex; the other is a central nervous system reflex which we have designated the enterogastric reflex (Thomas and Mogan, 1931).

As shown in vagotomized animals, the myenteric reflex extends its excitatory influence beyond the sphincter to the pyloric part of the stomach. The enterogastric reflex probably affects the whole stomach and was shown experimentally to inhibit the pyloric portion, including the sphincter. The peripheral fields of the two reflexes therefore overlap at the pylorus. Were it not for this overlapping we should have something resembling contrary innervation of the sphincter muscle and the muscle of the gastric wall, considered as antagonists; but this attractive conception is not supported by the experimental results.

The fact that moderately strong acid stimulation of the duodenum caused only momentary contraction of the pyloric sphincter followed by inhibition shows that the enterogastric reflex is capable of suppressing the myenteric reflex when both are excited simultaneously. With minimal stimuli the enterogastric reflex alone was evident; either the stimuli failed to attain threshold value for the myenteric mechanism or the reflex was inhibited to the extent that it failed to cause contraction of the sphincter.

This conception of the relationship between the two reflex mechanisms is supported by the experiments with anesthesia and after vagotomy. If our interpretation is correct, these conditions, by interfering with conduction in the arc of the enterogastric reflex, would diminish or entirely remove

its inhibitory influence on the myenteric reflex mechanism. As the results demonstrate, the conditions mentioned favor contraction of the sphincter in response to duodenal stimulation. It is conceivable that under other conditions, e.g., with a different type of stimulus or in certain pathological states, a similar change in the relation between the two reflexes may occur.

Should there be species differences, the central (enterogastric) reflex, which represents the higher type of regulatory mechanism, would probably be even more dominant in the more highly evolved species. It has the advantages of an apparently lower threshold and greater economy of effort. The enterogastric reflex mechanism has been demonstrated in the human species (McClure, Reynolds and Schwartz, 1920; Barsony and Egan, 1925).

The fact that the enterogastric reflex could be elicited in its characteristic form only when the vagus nerves were intact and that it was not influenced by division of the splanchnic nerves proves that a majority of the fibers involved are in the vagus. It is not necessary to assume that all of the fibers concerned are inhibitory in their peripheral action. Central inhibition of active excitatory neurons could cause most of the effects that we have classed as inhibition.

The residual inhibition that persisted after the vagus nerves were severed is probably not of sympathetic origin since it was not diminished in degree by division of the splanchnic nerves. On the other hand, it is opposite to the type of reaction expected when the myenteric reflex mechanism is excited. It may represent the reaction of this mechanism to the type or degree of stimulation that causes antiperistalsis as a part of the vomiting reflex. The fact that it could not be demonstrated with moderate stimuli practically eliminates it from consideration in connection with the normal regulation of gastric emptying.

Consideration of the factors concerned in the effect of duodenal stimulation on the emptying of the stomach. That duodenal stimulation delays the emptying of the stomach has long been known but whether the delay is due to closure of the pylorus or to inhibition of gastric peristalsis or to both remains in doubt. Pavlov (1902) expressed the opinion that, "Each time the intestine receives a portion of the acid contents of the stomach, a reflex act is set up which temporarily occludes the pyloric orifice and at the same time inhibits the propulsive movements of the organ." Cannon (1911) criticised this view because he was convinced that "the (gastric peristaltic) waves do not show from moment to moment marked variations of intensity," and he therefore attributed the effect solely to closure of the pylorus. Cannon's views were widely accepted and Pavlov (1910), in a later edition of his work which appeared after the first publication of Cannon's theory, omitted the reference to inhibition of peristalsis. Nevertheless, his suggestion has been revived from time to time in the more recent literature (Carlson and Litt, 1924; Katsch, 1926; Ivy, 1927).

That the pyloric sphincter is not necessary for a satisfactory regulation of the gastric output is indicated by the observation of von Mering (1897) that in dogs with resected pylorus the exit from the stomach of water, milk and sugar-peptone solutions occurred at intervals as usual and was neither slower nor faster than normal. Cannon and Blake (1905) found that in cats after pyloroplasty the exit of carbohydrate and protein food, although beginning earlier and progressing somewhat more rapidly than in normal animals, was still regulated satisfactorily. They expressed the opinion that the regulation was due to the rhythmic segmenting contractions of the duodenum.

On the other hand, experiments have been reported which seem to indicate that, normally, duodenal filling does not give rise to stimuli adequate to cause gastric inhibition. In the experiments referred to above Cannon and Blake observed that, in spite of filling of the small intestine, gastric peristaltic waves continued to course over the stomach as usual. Barsony and Egan (1925), although able to cause inhibition of the human stomach by placing acid in the duodenum, considered that the requisite stimuli were in excess of the normal.

Both of the studies mentioned were made by means of x-rays, with which the observers would probably not have been able to detect moderate grades of inhibition and would certainly have been unable to measure the force of the peristaltic contractions. Probably only complete or nearly complete cessation of gastric activity would have been noted. It is no more to be expected that the mechanism that regulates gastric motility will be stimulated to the extent of causing complete inactivity under normal circumstances than that the heart will normally be stopped by the mechanism that regulates the cardiac output. In each instance the regulatory reflex operates to prevent the stimulus from rising much above its threshold value. The methods that we have used provide a means of detecting and measuring moderate changes in the tonus of the pyloric sphincter and in the force of gastric peristalsis.

Our results indicate that the acid in the duodenum does not cause more than a temporary contraction of the pyloric sphincter unless the stimulus is sufficient to cause vomiting, and that during digestion considerable amounts of acid may be placed in the duodenum without increasing the tonus of the sphincter even momentarily. They show that stimulation of the duodenum that is not sufficient to increase the tonus of the sphincter may nevertheless be adequate to cause a considerable decrease in the force of the gastric peristaltic contractions. This evidence of a lower threshold for gastric inhibition deserves special emphasis because it is obvious that the weakest stimulus that will suffice to diminish materially the rate of emptying of the stomach will, by so doing, prevent the stomach from supplying stronger stimuli to the duodenum. Probably stimuli due to acid

sufficient to cause even a temporary increase in the tonus of the pyloric sphincter do not appear in the duodenum under normal circumstances.

We think that the effects of stimuli other than acid should be investigated before an attempt is made to evaluate finally the reactions of the pyloric sphincter to duodenal stimulation. However the theory that such reactions participate in controlling the emptying of the stomach involves also the view that acid is the effective stimulus; consequently, the whole conception of an acid control of gastric emptying by means of sphincteric reflexes appears to us to be without foundation in experimental fact.

On the other hand, the results with duodenal drainage leave no doubt that the accumulation of material within the intestine while the stomach was emptying provided stimuli adequate to cause inhibition of gastric peristalsis. It is reasonably certain, therefore, that gastric inhibition plays an important part in the effect of duodenal reflexes on the emptying of the stomach. In the case of reflexes due to acid we conclude that the delay in emptying is the result of gastric inhibition rather than closure of the pylorus.

SUMMARY. 1. A study of the reflexes aroused by injecting HCl into the stomach and acid and other substances into the duodenum and by the normal duodenal contents was made by graphic methods on unanesthetized dogs. Peristalsis in the pyloric portion of the stomach and the activities of the pyloric sphincter were recorded.

2. HCl injected into the stomach near the pyloric sphincter was without significant effect on the tonus of the sphincter.

3. HCl in the duodenum caused only temporary increase in the tonus of the pyloric sphincter followed by moderate relaxation and inhibition of the rhythmic contractions. When the stomach was full the primary contraction was less easily demonstrated and was frequently absent from the reaction.

4. Acid and other substances, including normal saline in sufficient volume, when placed in the duodenum caused inhibition of gastric peristalsis whether the stomach was full or empty.

5. The threshold for gastric inhibition was lower than for the temporary contraction of the sphincter due to acid in the duodenum, especially when the stomach was full.

6. The normal duodenal contents were found to act as adequate stimuli for the reflex mechanism causing inhibition of gastric peristalsis; draining the duodenum while the stomach was emptying regularly increased the force of the peristaltic contractions.

7. After section of the vagi, contraction of the pyloric sphincter was more marked and prolonged and inhibition less evident in the reactions to acid in the duodenum; some degree of inhibition of gastric peristalsis could still be obtained, but only with the use of stronger stimuli to the duodenum

than normally required and after a longer latent period. Duodenal drainage failed, in the vagotomized dogs, to influence gastric peristalsis.

8. Section of the splanchnics was without effect on any of the reflexes studied; this fact was interpreted as constituting a difference between them and reactions to noxious stimuli.

9. The results are interpreted as indicating the existence of two reflex mechanisms with their receptors in the duodenum, capable of influencing the tonus of the pyloric sphincter and the force of gastric peristalsis; a local excitatory reflex identified with the myenteric reflex, and a central inhibitory reflex over the vagus designated the enterogastric reflex.

CONCLUSION

The evidence, we think, is sufficient to warrant abandonment of the theory that the effect of duodenal stimulation on the emptying of the stomach is exerted mainly through reflex changes in the tonus of the pyloric sphincter. We propose instead the view that such regulation is accomplished principally through a reflex mechanism that governs the force of gastric peristalsis.

A corollary to this conclusion is that in the intact animal the emptying of the stomach is controlled to a greater extent through the central nervous system than has formerly been supposed.

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DEPRESSION OF THE ACTIVITY AROUSED BY A FLASH OF LIGHT BY APPLYING A SECOND FLASH IMMEDIATELY AFTERWARDS TO ADJACENT AREAS OF THE RETINA

GLENN A. FRY[1]

From the Department of Ophthalmology, Oscar Johnson Institute, Washington University, St. Louis

Received for publication March 26, 1934

In this paper is presented a quantitative study of a phenomenon which seems to involve some kind of interaction between neighboring retino-cortical paths at synapses either at the retina, or at the basal ganglia, or at the cortex.

The demonstration of the phenomenon consisted in keeping the whole field of vision dark except for the momentary exposure of three small, bright white areas, whose shape, size, and position with respect to each other are indicated in figure 1. Area b was exposed for a given duration, and after a lapse of time a and a' were exposed and it was determined at what duration they produced a sensation equally bright as that produced by b. The comparison of the brightness of a and a' with the brightness of b was made easy by two facts: 1, that the sensations produced by a and a' appeared simultaneously with that produced by b, and 2, that the areas, a and a', were strictly adjacent to b. But the comparison was hindered by the fact that a difference in hue obtained, a and a' being seen as greenish white and b as pinkish purple. To attempt an explanation of this difference in hue is beyond the scope of this paper. It is related to phenomena discussed in a previous paper (1933).

The timing of the exposures was executed in the following manner: By means of a head rest the right eye of the observer was fixed, as indicated in figure 2, at one of the conjugate foci of the lens, which was 16 inches away. That part of the face of the lens which was seen through the rectangular aperture, K, was of uniform brightness, 824 c. per sq. ft. The diameter of the artificial pupil was 2 mm. and the stimulus intensity was calculated to be 28,000 photons. Disk I' in figure 2 is a front view of disk I showing its position with respect to the aperture, K. Once every twenty-fifth revolution of the disk the illumination of the aperture was cut on by disks II and III just long enough for the open slits, B and A and A', to pass across and expose areas b, a and a'. The duration of the exposures was determined

[1] National Research Fellow in the Biological Sciences.

by the angular size of the slits and the speed of rotation, which was varied from 10 to 20 per second. The interval between the exposure of b and the exposure of a and a' was determined by the angular distance from the leading edge of slit B to the leading edges of slits A and A' and by the speed of rotation. The interval between successive presentations of the stimulus

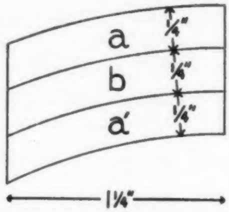


Fig. 1. Stimulus pattern

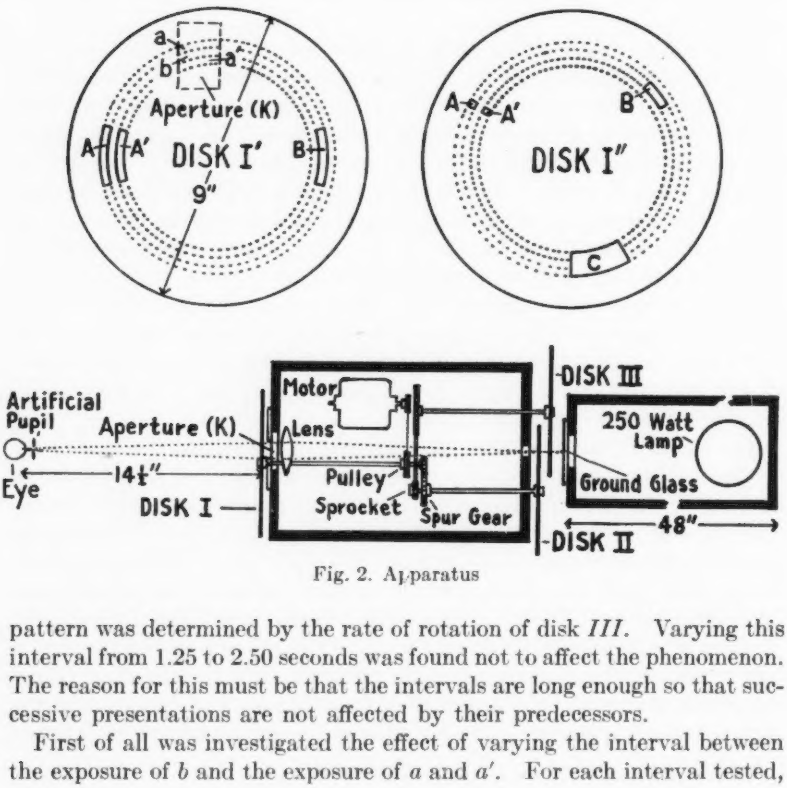


Fig. 2. Apparatus

pattern was determined by the rate of rotation of disk *III*. Varying this interval from 1.25 to 2.50 seconds was found not to affect the phenomenon. The reason for this must be that the intervals are long enough so that successive presentations are not affected by their predecessors.

First of all was investigated the effect of varying the interval between the exposure of b and the exposure of a and a'. For each interval tested,

the duration of exposure of a and a' was determined[2] which produced a sensation as bright as that produced by b, the duration of exposure of b being kept constant at 4σ. The results are plotted by circles in graph I in figure 3. The depressive effect increases as the interval increases, i.e., up to 150σ. At 150σ the duration of a and a' was too small to measure with accuracy. Beyond this point b appeared separate in time from a and a'; an illusion of movement was also present and a comparison of brightnesses was difficult.

The above experiment was varied by making the exposure of a and a' precede that of b. The duration of exposure of a and a' was kept constant at 4σ and it was determined what duration of exposure of b produced a

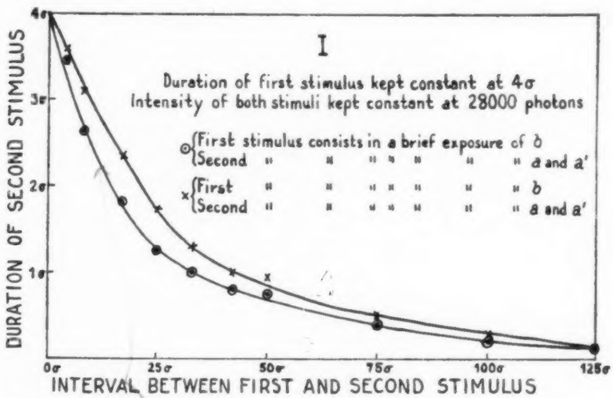


Fig. 3. For various intervals between the first and second stimulus, graph I shows the duration of the second stimulus which produces a sensation equally bright as that produced by the first, whose duration is kept constant.

sensation equally bright. The results are plotted by crosses in graph I in figure 3.

The fact that the second stimulus does not require as long duration as the first in order to produce a sensation equally as bright might indicate 1, either that the activity aroused by the first stimulus is depressed by the second, or 2, that the activity aroused by the second is enhanced by the first. But the following experiment proves that the former is actually the case. Area b was exposed 4σ, and 17σ after the beginning of this exposure a and a' were exposed 1.12σ. This asynchronous exposure of b and a and a' was alternated every 1.25 second with the simultaneous exposure of all

[2] The method consisted simply in trying various durations of a and a' (taken in steps of $.14\sigma$) until one was found at which a and a' were equal in brightness to b. This duration was so critical that an elaborate statistical method could not add enough to accuracy to warrant its use.

three areas.[3] Let us call this simultaneous exposure *c*. The sensations produced by *a*, *a'*, and *b* were equal in brightness. The problem for the observer consisted in determining the duration of stimulus *c* which produced a sensation equally bright as that produced by *a*, *a'* and by *b*. By the *method of limits* it was determined that this duration must lie between 0.70 and 1.26σ and must be very nearly equal to the duration of *a* and *a'*, namely 1.12σ. This indicates that the activity initiated by *b* is depressed by *a*

TABLE 1

Effect of varying the energy of stimulus b with respect to duration

INTENSITY OF STIMULI *a*, *a'* AND *b*	DURATION OF STIMULUS *b*	ENERGY OF STIMULUS *b*	DURATION OF STIMULI *a* AND *a'*	ENERGY OF STIMULI *a* AND *a'*
photons	σ	*sigma-photons*	σ	*sigma-photons*
28,000	4.0	112,000	1.96	54,880
	3.5	98,000	1.69	47,320
	3.0	84,000	1.47	41,160
	2.5	70,000	1.12	32,360
	2.0	56,000	0.77	21,560
	1.5	42,000	0.50	14,000
	1.0	28,000	0.39	10,920
	0.5	14,000	0.22	6,160

TABLE 2

Effect of varying the energy of stimulus b with respect to intensity

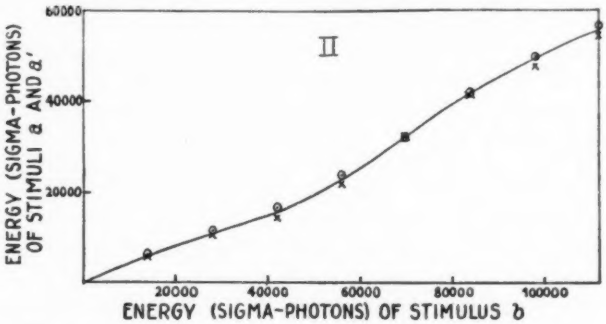
INTENSITY OF STIMULI *a*, *a'* AND *b*	DURATION OF STIMULUS *b*	ENERGY OF STIMULUS *b*	DURATION OF STIMULI *a* AND *a'*	ENERGY OF STIMULI *a* AND *a'*
photons		*sigma-photons*	σ	*sigma-photons*
28,000	4σ	112,000	2.03	56,840
24,500		98,000	2.03	49,735
21,000		84,000	1.96	41,160
17,500		70,000	1.82	31,850
14,000		56,000	1.68	23,520
10,500		42,000	1.60	16,800
7,000		28,000	1.60	11,200
3,500		14,000	1.75	6,125

and *a'*, and that the activity initiated by *a* and *a'* is not appreciably enhanced by *b*.

This conclusion is substantiated by the fact that the line through the circles in figure 3 falls lower than the line through the crosses. On the ground that the second stimulus depresses the first it should be expected

[3] Disk *I''* in figure 2 was rotated 10 times per second in front of the aperture (*K*, fig. 2) which was illuminated just long enough for slits *B* and *A* and *A'* to pass across in front of it, and 1.25 second later just long enough for slit *C* to pass across.

that the depression would be greater in the case of the circles than in the case of the crosses, because in the case of the circles the second stimulus is applied to a larger retinal area than the first, and *vice versa* for the crosses. Analogously it may be argued that if the first stimulus enhanced the second, the enhancement should be greater in the case of the crosses than in the case of the circles, but this is contrary to the facts.



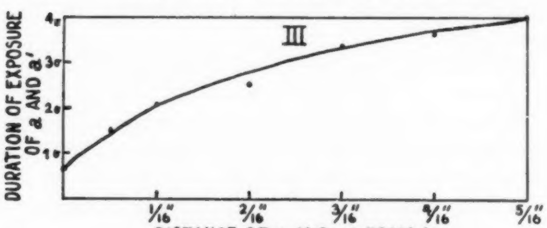


Fig. 4. In the case of graph II the exposure of b precedes the exposure of a and a' 17σ and this interval is kept constant. The graph shows the energy value of a and a' which produces a sensation equally bright as that produced by b. In the case of the crosses the intensity of b is kept constant at 28,000 photons and the energy of b is varied with respect to duration. In the case of the circles the duration of b is kept constant at 4σ and the energy of b is varied with respect to intensity.

In the case of graph III the exposure of b precedes the exposure of a and a' 50σ and this interval is kept constant. The distance of a and a' from b, however, is varied and the graph shows the duration of exposure of a and a' which produces a sensation equally bright as that produced by b, whose duration of exposure is kept constant at 4σ.

The depressive effect cannot be explained on Allen's hypothesis (1925) that stimulation of one part of the retina reflexly[4] depresses the sensitivity of surrounding parts to light, inasmuch as the second stimulus is applied

[4] Allen conceives that impulses arriving at the brain *via* afferent paths from one part of the retina give rise to impulses in efferent paths leading to surrounding parts of the retina, which depress the sensitivity of the retinal receptors to light.

to the retina after the first and cannot therefore have *reflexly* depressed the sensitivity of the retinal receptors to the first. What seems to happen is that the response of the retina to the first stimulus is considerably delayed and prolonged and overlaps in time the response to the second stimulus and is inhibited by it by some kind of interaction between retino-cortical pathways at synapses either at the retina, or at the basal ganglia, or at the cortex

It was aimed in the two following experiments to investigate the separate effects of varying the duration and intensity of the stimuli. In both cases b preceded a and a' 17σ. In one experiment the intensity of b was kept constant at 28,000 photons, and its duration was varied. For each duration tested, the duration of a and a' was determined which produced a sensation equally bright. The results are presented in table 1. In the second experiment the duration of b was kept constant at 4σ and its intensity was varied. For each intensity tested, the duration of a and a' was determined which produced a sensation equally bright. The results are presented in table 2. Graph II in figure 4 shows a comparison of the separate effects of varying the duration and intensity of stimulus b. The results are such as would be expected from the "reciprocity law" which states that the effect of a brief stimulus is determined by the product of its intensity and duration.

Hitherto areas a and a' have been kept strictly adjacent to b, but in the following experiment an attempt has been made to study the effect of varying the distance of a and a' from b. The exposure of b preceded the exposure of a and a' 50σ and this interval was kept constant. The brightnesses of a, a', and b were all kept constant at 824 c. per sq. ft. The distance of a and a' from b was varied and at the various distances the duration of exposure of a and a' was determined which produced a sensation equally bright as that produced by b, whose duration of exposure was kept constant at 4σ. The results are plotted in graph III in figure 4. They show that the depressive effect dies away as the distance increases, and disappears completely at about $\frac{5}{16}$ inch, which, in this case, subtends a visual angle of 1° 14′.

In the following experiment the wave-length composition of the light was varied. The ground glass screen illuminated by the 250 watt lamp was exchanged for a spectrometer, the image of whose slit was focused upon the artificial pupil. The stimulus intensity was kept constant at 1825 photons. Stimulus b preceded a and a' 50σ. The duration of b was kept constant at 4σ. Wave-lengths 20 mμ apart and ranging from 440 to 680 mμ were tested. For each wave-length the duration of a and a' was determined which produced a sensation equally as bright as that produced by b. This duration proved to be 1.8σ for all wave-lengths.

SUMMARY

In this paper it is shown that the activity aroused by a flash of light may be depressed by applying a second flash immediately afterwards to adjacent areas of the retina.

1. The effect of varying the interval between the two flashes was investigated and it was found that the depressive effect increased as the interval increased, i.e., up to 150σ. The experiment was not carried further for reasons pointed out, but it may be assumed that if the interval is made long enough, the depressive effect must begin to decrease and gradually disappear.

2. The effect of varying the intensity and duration of the flashes was investigated and it was found that a decrease in duration corresponded to a decrease in intensity. Only durations below 4σ were dealt with.

3. The effect of varying the distance apart of the areas stimulated was also investigated, and the results show that the depressive effect gradually dies out as the distance is increased.

4. Varying the wave-length composition of the light does not affect the phenomenon.

I wish to acknowledge thanks to Dr. P. W. Cobb for his helpful suggestions in regard to this investigation.

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THE EFFECT OF DIET ON THE DISTRIBUTION OF GLYCOGEN IN THE SKELETAL MUSCLE OF THE RAT[1]

MELVILLE SAHYUN, RAY SIMMONDS AND HOLBROOK WORKING

From the Department of Bacteriology and Experimental Pathology, the Department of Physiology and the Food Research Institute, Stanford University, California

Received for publication March 19, 1934

In this investigation the distribution of glycogen in various individual muscles is studied for rats that had been kept on different diets. Further, a comparison of the glycogen content among the individual muscles of the upper and lower right and left leg of the rat are reported.

Since the removal of different individual muscles requires careful manipulation and time, the animals were anesthesized. Sodium-ethyl-methyl-butyl-barbiturate, commonly called "nembutal," was employed, for in a previous communication Sahyun and Webster (1) had reported it to be satisfactory.

Glycogen determinations were performed according to the method of Sahyun (2, 3).[2]

Procedure. Healthy white rats were used in this investigation. The method of administering anesthesia and the removal of muscles are as previously outlined by Sahyun and Webster (1). It is to be noted, however, that the muscles were first denervated in order to prevent excitation, and great care was taken to free the individual muscles from fat, fascia and tendons as completely as possible.

The rats selected were all males of fairly uniform size and age. The ages of the rats employed in this work were between 165 and 235 days and the majority around 200 days. The average weight of the rat was about 210 grams. Lack of space prevents publication of all the data available.

In this connection we wish to express our thanks to Doctor Slonaker of the Department of Physiology of this University, who generously supplied us with all the animals. The rats were selected from five groups, each of which was kept on a different regimen. The composition of the different diets is as follows:

[1] This work is supported in part by a grant from Eli Lilly & Company, Indianapolis, Indiana.

[2] The author wishes to give herein due credit to Khan (4) for the use of the centrifuge in glycogen determination which inadvertently was omitted in his recent publication (3) on glycogen determination.

GROUP NUMBER	PROTEIN	FAT	CARBOHYDRATE
	per cent	*per cent*	*per cent*
I	10.3	12.2	77.5
II	14.2	14.2	71.6
III	18.2	15.9	65.9
IV	22.2	17.8	60.0
V	26.3	19.7	54.0

EXPERIMENTAL. 1. The effect of "nembutal" on the distribution of glycogen in the rat was studied. After a 24-hour fast the animal was intraperitoneally injected with the proper amount of the drug. As a rule 10 mgm. were found ample to completely anesthesize the animal, but there were instances in which larger quantities were necessary to bring about complete anesthesia. The skeletal muscles selected were the gastrocnemius and the quadriceps femoris. This experiment is divided into two parts: *a*, where the removal of the muscles of the left leg followed immediately the removal of those of the right; and *b*, where the muscles of left were allowed to stand for half an hour at room temperature while the animal was kept under anesthesia after the muscles of the right leg had been severed. Since our results confirm the previous findings of Sahyun and Webster (1) averages only are reported in the following table.

TABLE 1

Distribution of glycogen in the rat

The effect of "Nembutal" on the glycogen content of the skeletal muscle of the rat. Each figure represents the average of eight muscles.

	MILLIGRAMS GLYCOGEN PER 100 GRAMS TISSUE		
	Right	Left	
	0 hour	Immediately	0.5 hour
Gastrocnemius	338	349	
Quadriceps femoris	179	177	
Gastrocnemius	378		377
Quadriceps femoris	310		301

2. In this experiment the effect of various diets on the distribution of glycogen in the skeletal muscles of the rat was studied. Ten rats of each of the five groups previously mentioned were selected. The animals were fasted for 24 hours before the administration of the anesthetic. The skeletal muscles carefully dissected and used for glycogen determination are: the gastrocnemius, the quadriceps femoris, the triceps bracii and the

TABLE 2

Glycogen content—milligrams per 100 grams of tissue

Averages for 3 muscles (Quadriceps femoris, Pectoralis major, and Triceps bracii)

	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1	363	360	301	305	213	212	258	250	231	235
2	382	378	314	328	200	205	308	306	237	233
3	311	302	309	338	177	192	316	291	307	297
4	301	316	311	306	223	206	291	284	157	158
5	332	351	270	259	305	295	264	268	249	247
6	363	376	290	307	322	318	280	269	227	218
7	330	311	415	438	340	302	217	220	269	273
8	432	424	395	404	316	330	315	306	213	210
9	418	361	303	301	382	385	237	243	160	165
10			303	296	302	286	202	202	289	288
Average	351.7	352.2	322.4	327.1	278.0	273.1	263.6	260.9	233.9	232.4

Glycogen content of the gastrocnemius

	GROUP I		GROUP II		GROUP III		GROUP IV		GROUP V	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1	421	400	333	336	196	217	345	295	375	387
2	390	384	417	390	248	245	394	378	392	402
3	442	417	312	332	226	231	348	313	286	282
4	518	480	417	425	397	401	321	255	267	273
5	410	426	336	342	343	356	284	255	231	246
6	445	433	379	372	395	372	239	250	129	125
7	460	427	460	453	402	402	261	256	365	373
8	468	460	435	432	382	399	312	303	201	206
9	483	471	327	309	413	377	292	313	208	196
10			324	343	332	319	265	259	318	322
Average	448.6	433.1	374	372	333	331	306	287	277	281

TABLE 3

General averages of the glycogen content of the individual muscles of each group

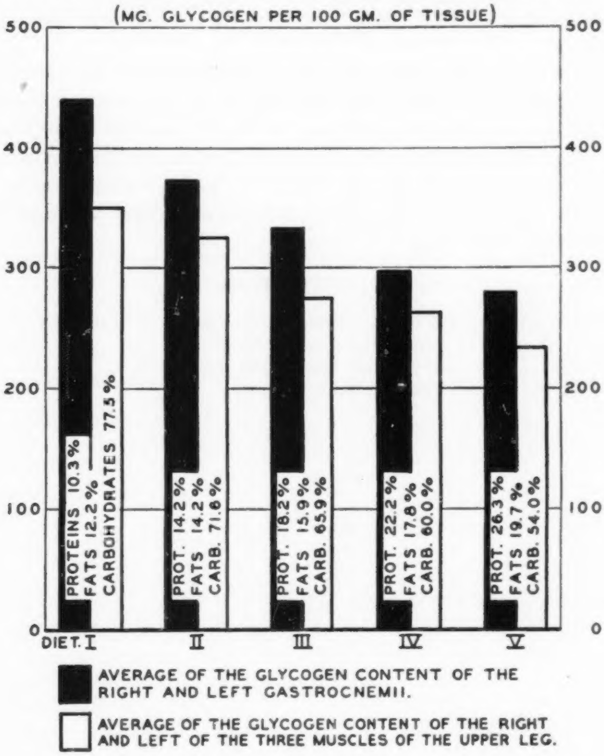
GROUP NUMBER	MILLIGRAMS GLYCOGEN PER 100 GRAMS OF TISSUE							
	Gastrocnemius		Quadriceps femoris		Triceps bracii		Pectoralis major	
	Right	Left	Right	Left	Right	Left	Right	Left
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
I	448	433	370	387	390	381	324	338
II	374	372	325	331	310	315	327	327
III	333	331	272	264	271	269	290	285
IV	306	287	257	258	275	265	275	268
V	277	281	233	231	226	225	241	240

pectoralis major. Glycogen determination was performed on each muscle separately. The muscles of the right leg were always removed first.

Since the data collected on the glycogen content of the individual muscles is voluminous, only the following are presented in the table here below:

a. The averages, for each animal, of the three muscles of the upper right and of the upper left leg, respectively; also the glycogen content of both the gastrocnemii. It is on these data that a statistical study was made.

THE EFFECT OF DIET ON THE GLYCOGEN CONTENT
OF THE SKELETAL MUSCLE OF THE RAT



b. The general averages of the glycogen content of the individual muscles of each group.

c. The general averages for all the muscles for each group. This is represented by the accompanying figure.

CONCLUSION

The uniformity of the results obtained from the glycogen determinations described above make two conclusions evident: 1. Glycogen content of the muscles of the rat under the conditions of the experiments tends to vary directly with the percentage of carbohydrate in the diet, being over 50 per cent greater in the animals on a high carbohydrate diet than in animals on a high protein diet.

2. Glycogen content tends to run about 18 per cent higher in the gastrocnemius than in the three muscles of the upper leg. Appropriate statistical tests bear out these conclusions and lead to the following additional conclusions:

3. The experimental errors encountered arose from individual differences among the animals rather than from errors in glycogen determinations.

4. There is substantial evidence of higher glycogen content in the muscles of the right leg than in muscles of the left, perhaps attributable to differences existing in the living animal or perhaps attributable solely to greater loss of blood from muscles of the left leg, which was uniformly dissected after the right leg.

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